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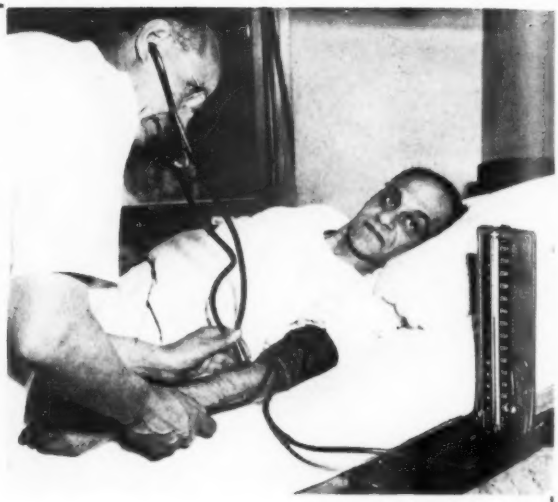

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

MARCH, 1950

ORIGINAL ARTICLES

THE SURGICAL TREATMENT OF SPONTANEOUS AND TRAUMATIC INTRACEREBRAL HEMORRHAGE

By Francis C. Grant, M.D.
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AND
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This report is based on 14 verified cases of intracerebral hemorrhage operated on at the Hospital of the University of Pennsylvania or Graduate Hospital of the University of Pennsylvania. Five of these cases were traumatic and 9 were spontaneous in origin. Frequently the preoperative diagnosis was doubtful.

However, in a patient, whether hypertensive or not, who develops sudden hemiparesis, headache, or unconsciousness, and these symptoms then progress, the possibility of an intracerebral hemorrhage must be considered. This is particularly true in the cases in which trauma has been a factor. In a post traumatic case, with localized and progressive symptoms, exploration of the involved area is of course indicated. In the absence of extra- or subdural bleeding, the possibility of an intracerebral hemorrhage must be kept in mind and the cortex needled, lest a subcortical hemorrhage be overlooked.

In this group 2 cases were hypertensive. In the 4 fatal cases, 3 came to autopsy and in none of these was an aneurysm discovered. In the 10 cases that recovered, the precise etiology is uncertain. Syphilis and brain tumor, however, can be eliminated as a possibility in every case.

The early publication of Charcot and Bouchard[4], in 1868, was the first tending to place the etiology of intracerebral hemorrhage on aneurysms. Although this theory of numerous small "berry" aneurysms is not generally accepted, it was their work which focused the general attention on aneurysms as a possible etiology. Other authors still believe that aneurysms of a different type may be a causative factor in intracerebral hemorrhage. Rowbotham[14] feels that when involving the circle of Willis, some congenital aneurysms frequently become attached to cerebral substance, rupturing into the brain itself and forming an intracerebral hematoma. Scheinker[16], Dav-

is[8], Sachs[15], and Craig and Adson[5] have all stressed the probability of the venous origin of these hemorrhages.

In cases of trauma, capillary hemorrhages coalescing into a single large hemorrhage have been suggested by Evans and Scheinker[9] as one of the more common sources. Bollinger's[1] original theory of late traumatic apoplexy emphasized the importance of softening associated with focal necrosis and the gradual formation of hemorrhage in a period of 1 to 8 weeks following trauma. Cases of angiomatous rupture leading to intracerebral hemorrhage have been cited by Davidoff[7], Michael and Levin[11], and Dandy[6].

The object of this report is to emphasize the diagnostic possibilities of intracerebral hemorrhage preoperatively and to point out the comparative ease of evacuation with good operative result. The surgical treatment of cerebral hemorrhage should increase in importance as our knowledge of the control of hypertension improves.

Clinical Data. The age incidence appears to be relatively unimportant. The range in traumatic cases was 27 to 43 years, with an average age of 35.4 years. All 5 of these patients were males. In the nontraumatic group the range was 19 years to 66 years, with an average of 44.3 years. Five females and 4 males comprised this group. From these figures it is obvious that the nontraumatic cases tend to occur in an older age group. It is also apparent that this type of hemorrhage is not a frequent occurrence in children or adolescents. In a group of 29 cases of traumatic intracerebral hemorrhage collected by Browder and Turney[2], the range was 28 to 70 years, with an average of 41.3 years.

In our group of patients it is obvious that intracerebral hemorrhage is not a cause of immediate death. The interval from onset of hemorrhage until operation varied greatly in these patients. The shortest interval was 1½ hours. Several patients gave histories suggestive of hemorrhage more than 5 months prior to operation. Obviously these cases were well encapsulated by the time they came to operation, and the brain had ample time to compensate for the lesion. We have not found any cases with death occurring in less than 2 hours following hemorrhage.

Consciousness. Sudden loss of consciousness was present as the initial symptom in 10 cases (Table 1). This was found in 4 out of 5 traumatic cases and present in 6 out of 9 nontraumatic cases. In the 1 traumatic case in which this was not the initial symptom there was a progressive stupor following the trauma. Naffziger and Brown[12] noted unconsciousness as an initial symptom in 2 out of the 3 traumatic cases they described. Penfield[13] has emphasized the importance of rapid and persisting coma, or slowly advancing stupor ending in coma, as the differential point in favor of cerebral hemorrhage rather than cerebral thrombosis. Cadwalader[3] pointed out that the less severe the disturbance of consciousness, the more likely that it is caused by softening and not by hemorrhage. Generalized convulsions followed by unconsciousness were an early symptom in 2 nontraumatic cases and were noted several weeks following trauma in 1 patient.

TABLE 1. PREDOMINANT NEUROLOGIC FINDINGS IN INTRACEREBRAL HEMORRHAGE

14 Cases

	Traumatic (5)	Non-traumatic (9)
Hemiparesis	5 (100%)	6 (67%)
Initial Unconc.	4 (80%)	6 (67%)
Convulsions	1 (20%)	2 (22%)
Severe Headache	0	5 (56%)

Motor Weakness. A definite hemiparesis or hemiplegia was present in 11 patients. In all of the traumatic cases

this was found to some degree. Five patients had a facial weakness in addition to the hemiparesis. In the 3 patients without hemiparesis or hemiplegia, 1 had paresthesia of both hands, and 2 patients had no motor or sensory findings. Davis[8] states that in intracerebral hemorrhage due to venous bleeding, there is a preponderance of focal signs over those of increased intracranial pressure, which is the reverse of epidural and subdural hemorrhage.

Visual and Pupillary Changes. Four patients had some degree of papilledema varying from blurred discs occurring in 2 patients, to 1 patient with 3 to 4 diopters of choking bilaterally. In 7 patients, pupillary changes of one form or another were present. The pupils were bilaterally dilated in 3 patients, unilaterally dilated in 1 patient, failed to react to light in 2, and were bilaterally constricted in 1 patient. These findings would indicate that pupillary signs are usually not of a localizing or consistent nature. Visual fields were studied in 5 patients. Three of these showed an homonymous hemianopia; 1 showed generally contracted fields, and in 1 patient the fields were normal. The state of consciousness and the brief interval between hospital admission and operation explains why visual fields were not done more frequently.

Spinal Fluid Findings. Spinal fluid pressures were measured in 11 patients and in 6 of these the pressure was abnormally elevated (that is, greater than 200 mm. of water, with the patient recumbent) (Fig. 1.) The spinal fluid was pink or bloody in 6 of these cases, and slightly xanthochromic in 2 patients. In 3 cases of spontaneous intracerebral hematoma, Scott[17] found that the cerebrospinal fluid was either "pink" or xanthochromic on admission or became xanthochromic before operation. He also noted an increase in total proteins and elevation of the cerebrospinal fluid pressure in all cases. Penfield[13], likewise, found that in his cases the cerebrospinal fluid was xanthochromic although the hematoma was strictly intracerebral.

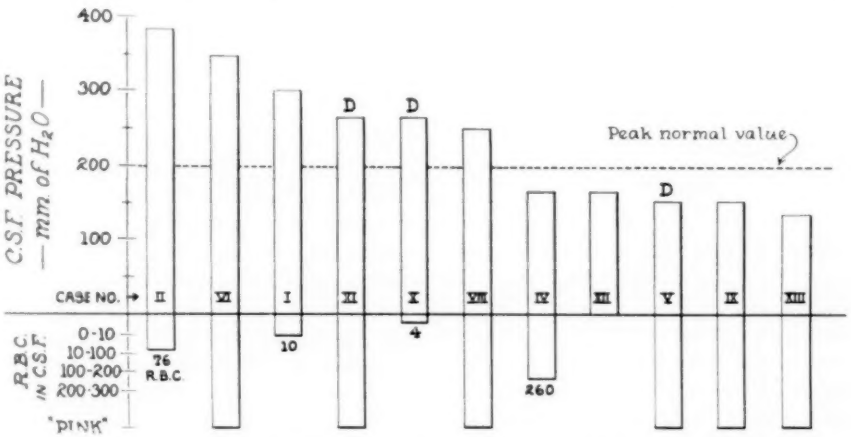

Fig 1.—Results of lumbar puncture in intracerebral hemorrhage (11 patients).

Diagnosis and Etiology. The diagnosis of intracerebral hemorrhage is often difficult due to its frequent resemblance to mass lesions and especially to subdural or extradural hemorrhage. It is significant that 4 of our cases were diagnosed preoperatively as

brain tumor; 4 were diagnosed as subdural hemorrhage, 3 as extradural hemorrhage, 1 as brain abscess, and 2 were correctly diagnosed as intracerebral hemorrhage. In all cases the hemorrhage was found at operation, with the exception of 1 which occurred in the right optic thalamus. There was no single predominant site of hemorrhage in this series. However, in 5 patients the bleeding occurred into the temporal lobe, including 2 of the 5 traumatic patients (Table 2). In 3 patients the site of hemorrhage was frontal; 2, frontoparietal; 2, parietal; 1, parietooccipital; and 1, thalamic.

TABLE 2. LOCATION OF INTRACEREBRAL HEMORRHAGE IN 14 CASES

Temporal	5
Frontal	3
Parietal	2
Fronto-parietal	2
Parieto-occipital	1
Thalamus	1

Other authors have also found the majority of hemorrhages to be in the temporal lobes. In Scott's[17] 3 cases of spontaneous hemorrhage, all were located in the temporal lobe. Hamby[10] noted 3 cases of temporal lobe hemorrhage out of 11 cases unassociated with tumor. One of Rowbotham's[14] 2 cases of aneurysm ruptured into the temporal lobe. Two of the 3 cases of "late traumatic apoplexy", presented by Naffziger and Brown[12], were temporal. Of 9 cases presented by Craig and Adson[5] 4 hemorrhages were found in the temporal lobes, and Browder and Turney[2] found 11 out of 29 traumatic cases to have lesions in the temporal lobe. Obviously these hemorrhages may occur in any part of the brain, but tend to occur more frequently in the temporal areas. On the average, approximately one-third to one-half of all intracerebral hemorrhage is located in the temporal lobe.

Hypertension is a frequent etiologic factor in this type of hemorrhage. However, in only 2 of our patients was there a history of abnormally high blood pressure.

Because the cases we have presented were all operative cases with only occasional postmortem examination, the precise cause of these hemorrhages must remain in doubt. Obviously, in some of the younger age group, an aneurysm would seem to be a plausible cause. However, an actual aneurysm was never discovered at operation.

Operation and Results. The time interval from initial symptoms of hemorrhage until operation had no important bearing on the postoperative result. Likewise, the type of operation did not affect the eventual result. In 7 cases the blood was evacuated through a burr hole, and in 6 cases an osteoplastic craniotomy was required. Two out of 5 (40%) traumatic cases were fatal, as were 2 out of 9 (22%) nontraumatic cases. In a small series of cases such as this, it is difficult to say unequivocally that the traumatic type has a graver prognosis. However, the additional effects of contusion cannot be minimized. The overall mortality, in this series of 14 cases, was 28.6%.

The lack of more cases of hemorrhage associated with hypertension makes it impossible to evaluate this type of treatment in apoplexy. Certainly it is worth while to attempt evacuation of any known intracerebral hemorrhage not involving the brain stem, in spite of a history of high blood pressure.

Summary. Nine cases of spontaneous hemorrhage and 5 cases of traumatic hemorrhage were thoroughly studied with respect to preoperative symptomatology, operative treatment, and postoperative results.

A localizing hemiparesis and initial unconsciousness were the commonest neurological findings in both types of hemorrhage. The operative diagnosis

was made by means of a burr hole in most cases, followed by aspiration of the hemorrhage through the burr hole, and occasionally by a wider exposure following craniotomy.

These hemorrhages may occur in any lobe but are known to be most frequent in the temporal lobes. In this series, 40% were found in the temporal lobes.

Since this report, 1 additional case of spontaneous intracerebral hemorrhage has been successfully operated upon and discharged from the hospital. This was a 65-year old, white male diagnosed preoperatively as a probable glioma. Operation revealed a large hemorrhage in the right temporal lobe.

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ANGIOCARDIOGRAPHY IN THE DIAGNOSIS OF CARDIOVASCULAR SYPHILIS*

By George E. Peabody, M.D.

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Angiocardiography is a Roentgen technique which aids in confirmation or exclusion of the clinical diagnosis of cardiovascular syphilis. By this procedure the cardiac chambers and associated great vessels can be roentgenologically visualized at appropriate intervals following the rapid intravenous injection of a contrast substance. Although the active syphilitic process underlying and preceding cannot thus be revealed, early gross anatomical changes resulting therefrom are rendered clearly perceptible. Of particular assistance in the clinically latent period of late syphilis wherein clinicopathological experience has indicated a high percentage to have syphilis of the cardiovascular system[3], angiocardiography may prove to be an important diagnostic procedure in the early diagnosis of cardiovascular syphilis.

In the past, the best available means of ascertaining the presence of cardiovascular syphilis has been through history-taking, physical examination, and chest roentgenography. Since it is most essential to establish the diagnosis early, thus gaining a better prognosis by promoting more intensive antisyphilitic therapy and prompt work restriction, it is unfortunate that early cardiovascular syphilis (that is, uncomplicated aortitis and small aneurysms of innominate artery and aorta) is fundamentally symptomless, without signs, and not apparent by chest roentgenography. Wilson[11], in a review of 211 cases of syphilitic aortitis, concluded that since uncomplicated syphilitic aortitis is asymptomatic, criteria based on symptoms are unreliable in making an early diagnosis. The presence of an aortic systolic murmur, and prolongation and metallic character of the second aortic sound have been considered helpful in indicating possible syphilitic aortitis, but White[10] has pointed out that such signposts are of little real value. Steel[7] in a study of 40 proved cases of syphilitic aortitis, decided that the roentgenological findings of dilatation and density in the syphilitic aorta were indistinguishable from other similar lesions of the aorta. Sussman[9], while analyzing the importance of roentgenography in this respect, stated that

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TABLE 1.—RESULTS OF ANGIOCARDIOGRAPHY CORRELATED WITH PHYSICAL EXAMINATION AND CHEST ROENTGENOGRAPHY IN 93 PATIENTS INVESTIGATED FOR CARDIOVASCULAR SYPHILIS AT THE NEW YORK HOSPITAL

Case and No.	*NYH No.*	*Serological Tests for Syphilis*	*Phys. Exam. for Cardiovascular Syphilis*	*Conventional Chest Roentgenography and Fluoroscopy*	*Angiocardiogram*	*Final Diagnosis*
1 ES	266 082	Pos.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
2 AF	378 475	Pos.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
3 SD	496 974	Neg.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
4 AA	306 716	Neg.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
5 GF	398 694	Pos.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
6 CJ	517 380	Pos.	Pos.	Neg.	Syph. aortitis	Syph. aortitis
7 RH	482 535	Pos.	Pos.	Neg.	Syph. aortitis & saccular aneurysm of desc. aorta	Syph. aortitis & saccular aneurysm of desc. aorta
8 GM	516 163	Pos.	Neg.	Possible aneurysm of innom. art.	Syph. aortitis, no aneurysm of innom. artery	Syph. aortitis
9 MC	109 215	Pos.	Neg.	Neg.	Syph. aortitis	Syph. aortitis
10 MM	396 676	Neg.	Neg.	Neg.	Syph. aortitis, aneurysm of innom. artery	Syph. aortitis, aneurysm of innom. artery
11 AW	502 714	Neg.	Neg.	Neg.	Syph. aortitis	Syph. aortitis
12 JH	126 896	Neg.	Neg.	Neg.	Syph. aortitis	Syph. aortitis
13 RV	511 373	Pos.	Neg.	Neg.	Syph. aortitis	Syph. aortitis
14 CS	289 491	Pos.	Pos.	Mediast. mass	Syph. aortitis & saccular aneurysm of aorta	Syph. aortitis & saccular aneurysm of aorta
15 JM	196 563	Neg.	Pos.	Mediast. mass	Syph. aortitis & saccular aneurysm of aorta	Syph. aortitis & saccular aneurysm of aorta
16 IG	517 285	Pos.	Pos.	? Syph. aortitis	Neg.	Late latent syph.
17 NM	71 696	Neg.	Pos.	? Syph. aortitis	Neg.	Late latent syph.
18 LC	280 562	Pos.	Pos.	? Syph. aortitis	Neg.	Late latent syph.
19 BY	422 950	Pos.	Pos.	? Syph. aortitis	Neg.	Tabes dorsalis
20 MM	124 729	Pos.	Pos.	? Syph. aortitis	Neg.	Late latent syph.
21 LR	407 209	Pos.	Pos.	? Syph. aortitis	Neg.	Late asymptomatic neurosyphilis
22 MG	512 041	Neg.	Pos.	? Aneurysm of aorta	Neg.	Bronchogenic cyst of mediastinum
23 FH	501 657	Neg.	Neg.	? Aneurysm of aorta	Neg.	Tumor of right lung
24 EC	68 370	Pos.	Neg.	? Aneurysm of aorta	Neg.	Late latent syphilis, tumor of mediastinum
25 GK	479 987	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.*
26 AM	510 975	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
27 BH	100 937	Neg.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
28 JR	286 584	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
29 LR	314 124	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
30 WS	102 424	Pos.	Pos.	Neg.	Syph. aortitis	Tabes dorsalis, primary optic atrophy, syph. ht. dis. with A.I.
31 AS	474 856	Pos.	Pos.	Neg.	Syph. aortitis	Late asympt. neurosyph. & syph. ht. dis. with A.I.
32 AC	185 540	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
33 DB	346 042	Neg.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
34 CD	447 799	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
35 OL	421 984	Pos.	Pos.	Neg.	Syph. aortitis & aneurysm of aorta	Syph. ht. dis with A.I. & aneurysm of aorta
36 RH	430 650	Pos.	Pos.	Neg.	Syph. aortitis & aneurysm of aorta	Syph. ht. dis. with A.I. & aneurysm of aorta
37 EC	166 584	Pos.	Pos.	Neg	Syph. aortitis & aneurysm of aorta	Syph. ht. dis. with A.I. & aneurysm of aorta
38 MB	525 951	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
39 GT	521 733	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
40 AG	530 348	Pos.	Pos.	Neg.	Syph. aortitis	Syph. ht. dis. with A.I.
41 AS	396 163	Pos.	Pos.	Neg.	Syph. aortitis & aneurysm of innom. artery	Syph. ht. dis. with A.I. & aneurysm of innom. artery
42 GF	253 344	Pos.	Pos.	? Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
43 CR	436 435	Pos.	Pos.	? Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
44 AS	193 824	Pos.	Pos.	? Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
45 AM	252 085	Neg.	Pos.	? Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
46 JC	228 747	Pos.	Pos.	"Mediastinal mass" or aortic aneurysm	Syph. aortitis & aneurysm of aorta	Syph. aortitis and aneurysm of aorta
47 AT	491 616	Pos.	Hypertension	Neg.	Slight smooth dilatation of aorta	Late latent syph. and hyper. cardiovasc. dis.
48 AF	521 063	Pos.	Hypertension Pos.	Neg.	Slight smooth dilatation of aorta	Late asympt. neurosyph. & hyper. cardiovasc. dis.
49 MG	506 977	Pos.	Pos.	Aneurysm of aorta	Syph. aortitis	Syph. aortitis
50 BS	255 612	Neg.	Pos.	Syph aortitis	Syph. aortitis	Late asympt. neurosyph. & syph. aortitis
51 OL	3 679	Pos.	Pos.	Syph. aortitis	Syph. aortitis	Syph. aortitis
52 EJ	262 950	Pos.	Pos.	Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
53 WC	400 546	Pos.	Pos.	Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
54 MH	216 453	Pos.	Pos.	Syph. aortitis	Syph. aortitis	Syph. ht. dis. with A.I.
55 JN	129 801	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Meningovasc. syph., syph. aortitis, & aneurysm of aorta

TABLE 1. (*Continued*)

Case and No.	NYH No.	*Serological Tests for Syphilis*	*Phys. Exam. for Cardiovascular Syphilis*	*Conventional Chest Roentgenography and Fluoroscopy*	*Angiocardiogram*	*Final Diagnosis*
56)FM	469 028	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Syph. aortitis and aneurysm of aorta
57)FS	476 034	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Syph. aortitis and aneurysm of aorta
58)AG	508 699	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Syph. ht. dis. with A.I. & aneursym of aorta
59)WF	206 926	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Syph. ht. dis. with A.I. & aneurysm of aorta
60)GT	454 982	Pos.	Pos.	Aortic aneurysm	Syph. aortitis & aortic aneur.	Syph. ht. dis. with A.I. & aneurysm of aorta
61)MS	495 140	Pos.	Neg.	Neg.	Neg.	Early asympt. neurosyph.
62)LP	500 898	Pos.	Neg.	Neg.	Neg.	Early latent syph.
63)LK	501 265	Pos.	Neg.	Neg.	Neg.	Early latent syph.
64)IN	500 641	Pos.	Neg.	Neg.	Neg.	Early latent syph.
65)MF	411 689	Pos.	Neg.	Neg.	Neg.	Early latent syph.
66)JW	503 973	Pos.	Neg.	Neg.	Neg.	Early latent syph.
67)RG	505 042	Pos.	Pos.	Neg.	Neg.	Early latent syph.
68)JD	86 213	Pos.	Neg.	Neg.	Neg.	Late latent congenita syph.
69)MC	74 460	Neg.	Pos.	Neg.	Neg.	Late meningovasc. syph.
70)RR	373 368	Pos.	Pos.	Neg.	Neg.	Tabes dorsalis
71)ND	278 141	Neg.	Pos.	Neg.	Neg.	Late asympt. neurosyph.
72)JL	336 973	Pos.	Pos.	Neg.	Tortuous loop in desc. aorta, arteriosclerosis	Late latent syph.
73)HW	31 678	Pos.	Pos.	Neg.	Neg.	Tabes dorsalis
74)JD	276 149	Pos.	Pos.	Neg.	Neg.	Late meningovasc. syph.
75)BL	507 264	Pos.	Pos.	Neg.	Neg.	Late latent syph.
76)MU	201 471	Neg.	Pos.	Neg.	Neg.	Late benign syph. (treated)
77)EF	155 155	Pos.	Pos.	Neg.	Neg.	Late latent syph.
78)CA	522 709	Pos.	Pos.	Neg.	Neg.	Late latent syph.
79)HR	331 157	Pos.	Pos.	Neg.	Neg.	Late asympt. neurosyph.
80)DK	418 026	Pos.	Pos.	Neg.	Neg.	Tabes dorsalis
81)JP	523 344	Pos.	Pos.	Neg.	Neg.	Late latent syph.
82)RM	404 769	Pos.	Neg.	Neg.	Neg.	Late latent syph.
83)PP	79 659	Pos.	Neg.	Neg.	Neg.	Late latent syph.
84)JS	165 826	Pos.	Neg.	Neg.	Neg.	Late latent syph.
85)CR	501 487	Pos.	Neg.	Neg.	Neg.	Late latent syph.
86)LB	235 836	Pos.	Neg.	Neg.	Neg.	Late asympt. neurosyph.
87)JM	514 722	Pos.	Neg.	Neg.	Neg.	Late latent syph.
88)WR	392 041	Pos.	Neg.	Neg.	Neg.	Late latent syph.
89)ES	494 891	Pos.	Neg.	Neg.	Neg.	Late latent syph.
90)HK	501 196	Neg.	Neg.	Neg.	Neg.	Late latent syph.
91)CP	500 671	Pos.	Neg.	Neg.	Neg.	Late latent syph.
92)BJ	420 642	Pos.	Neg.	Neg.	Neg.	Late asympt. neurosyph.
93)SM	521 391	Pos.	Neg.	Neg.	Neg.	Late latent syph.

*A.I.—Aortic Insufficiency

whereas definite deviations from the normal in the aorta were indicative of disease, mensuration in chest roentgenography did not allow for precise determination of minor deviations, particularly in the ascending portion. He reported that Roentgen examination, as such, did not prove particularly valuable in the early diagnosis of syphilitic aortitis, and that the value of the examination had been overestimated. White[10] has concluded that "the early diagnosis of cardiovascular syphilis is practically impossible (and) that advanced and often rapidly fatal aortic disease is present" before diagnosis is possible.

Thus, angiocardiography, by allowing for an exact study of otherwise obscure cardiovascular structures, fills a real need in the accurate and early diagnosis of cardiovascular syphilis.

Method. A series of 93 patients at the New York Hospital, with or without symptoms or signs indicative of cardiovascular syphilis, were studied angiocardiographically according to the method of Robb and Steinberg,[1] the technique of which has changed little since the earliest published descriptions[2,5,6]. Following history-taking, physical examination, laboratory study, and routine" chest

TABLE 2.—DIAGRAMATIC SUMMARY OF RESULTS OF TABLE 1

93 Cases Total

2 without syphilis
7 with early syphilis
1 with congenital syphilis

83 cases with late syphilis

49 cases with cardiovascular syphilis

34 which could be diagnosed without angiocardiography

3 with syphilitic aortitis diagnosed by combination of physical examination and chest roentgenogram and confirmed by angiocardiography.

27 with aortic insufficiency allowing for clinical diagnosis of syphilitic heart disease, corroborated by angiocardiography.

4 with aneurysm of the aorta perceptible on physical examination and chest roentgenography and corroborated by angiocardiography.

15 detectable only by angiocardiography

2 with syphilitic aortitis and aortic aneurysm, not detected as such by physical examination and chest roentgenography.

6 with syphilitic aortitis with no physical findings and negative chest roentgenogram.

7 with syphilitic aortitis with suggestive physical findings and negative chest roentgenogram.

34 cases without cardiovascular involvement

(21 with physical findings indicating possible cardiovascular syphilis.)

(7 with syphilitic aortitis suggested by chest roentgenogram, but ruled out by angiocardiography.)

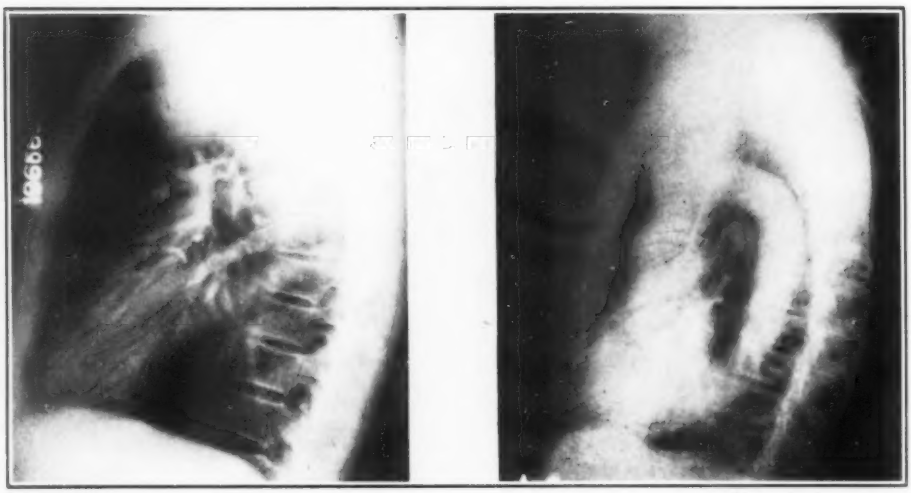
roentgenography, multiple contrast films of the cardiac chambers and associated great vessels were obtained from a distance of 6 feet immediately after intravenous injection of a contrast substance.

Studies were made in the left anterior oblique projection, allowing for an open view of the entire aortic arch. Results of aortic examination were correlated with measurements conducted on 100 normal aortas (including those showing atherosclerotic changes) which revealed the maximum caliber of the mid-ascending aorta in non-syphilitic individuals to be 38 mm[1]. A study of 61 angiocardiographically visualized aortas, previously reported, revealed that certain structural changes may be found as a consequence of syphilitic aortitis[8]: 1, Dilatation of the mid-ascending aorta beyond the upper limit of 38 mm. 2, Irregularity of the lumen of the ascending aorta. 3, Variations in thickness of the aortic wall. 4, Calcification of the ascending aorta which may be accurately localized with angiocardiography. 5, Tortuosity of the aorta.

Although dilatation of the aorta is not alone pathognomonic of syphilitic aortitis, and may be caused also by hypertension, atherosclerosis of the aorta, rheumatic aortic valvular insufficiency, and coarctation of the aorta, it would appear that this complex of findings is indicative of syphilitic aortitis and nothing else.

Aneurysms of the aorta and adjacent arteries are similarly revealed by angiocardiography.

In this report the results of angiocardiography are correlated in each case with

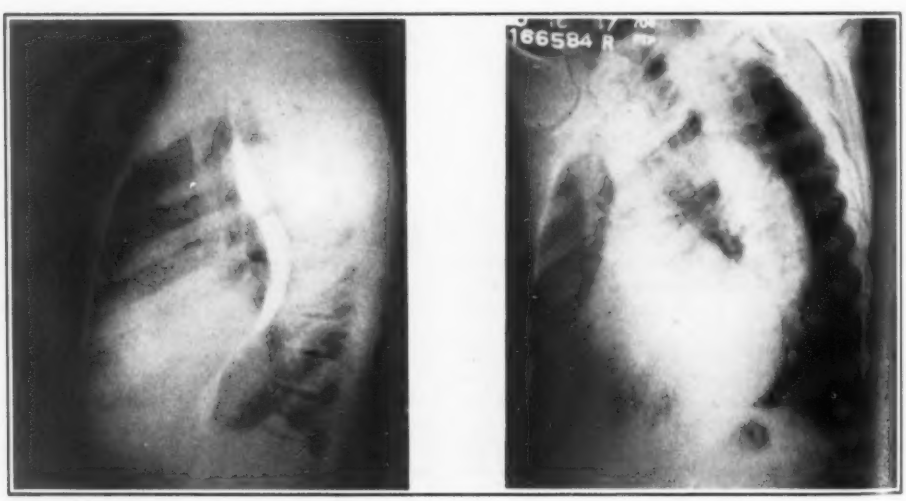


A *B*

CASE 15 (J.M.)

FIG. 1A. A 53-year old male with the clinical diagnosis of asymptomatic neurosyphilis. Lateral chest roentgenogram revealed a superior mediastinal mass anterior to the trachea interpreted to represent retrosternal thyroid tissue.

FIG. 1B. Angiocardiogram in left anterior oblique projection revealed opacification of a dilated irregular thoracic aorta (45 mm. in mid-ascending portion), and outlined an aneurysm arising from the proximal portion of the aortic arch. On frontal study, this was identified as an aneurysm of the innominate artery.



A *B*

CASE 37 (E.C.)

FIG. 2A. A 57-year old male with the clinical diagnosis of syphilitic heart disease with aortic insufficiency. Conventional lateral roentgenogram revealed only generalized cardiac enlargement.

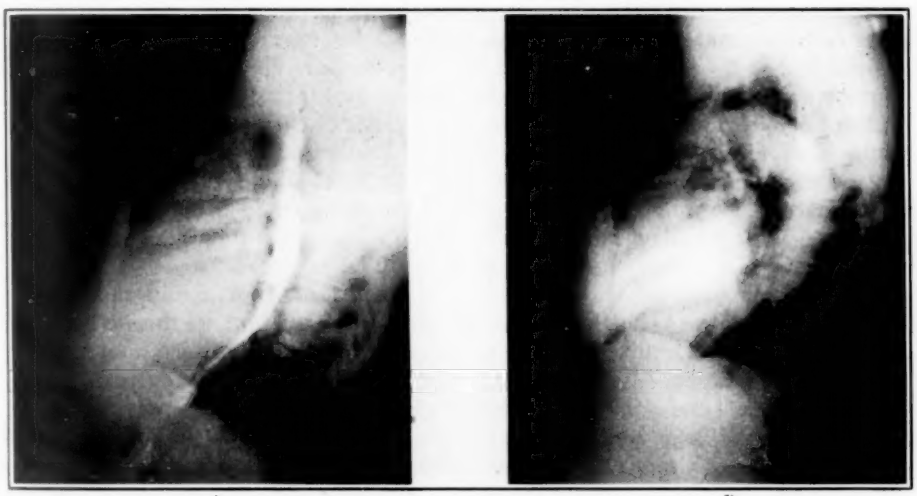
FIG. 2B. Angiocardiogram in left anterior oblique projection revealed irregularity and dilatation of the ascending aorta. A 7.5 cm. aneurysm of the sinus of Valsalva was clearly opacified. This was previously unsuspected.

the results of physical examination and chest roentgenography.

Results. Ninety-three patients were studied by angiocardiography and the results compared with physical findings and chest roentgenography in each case. Results are detailed in Table 1 and summarized in Table 2.

Eighty-three of the 93 had late acquired syphilis and thus potential cardiovascular syphilis. Of the 83 late syphilis patients, 49 were found to have cardiovascular syphilis with aortitis, aneurysm, or aortic insufficiency, 34 of which could be diagnosed without the aid of angiocardiography. Of these 34 cases, 27 had aortic insufficiency indicating the diagnosis in itself, 4 had aneurysms of the aorta perceptible by physical examination and chest roentgenography, and 3 had syphilitic aortitis detected in the same manner. The remaining 15 of the 49 cardiovascular syphilis patients would have been diagnosed as late latent syphilis without the benefit of angiocardiography.

Intravenous contrast study established the diagnosis in 13 cases of uncomplicated syphilitic aortitis in which conventional chest roentgenography was negative, and in 2 cases with syphilitic aortitis and aneurysm shown by chest Roentgen-ray to have only a mass in the mediastinum (Figure 1).



A *B*

CASE 43 (C.R.)

FIG. 3A. A 55-year old female with the clinical diagnosis of syphilitic heart disease with aortic insufficiency. Conventional lateral roentgenogram revealed a calcified ascending aorta which seemed to be dilated.

FIG. 3B. Angiocardiogram in the left anterior oblique projection revealed opacification of a widely dilated irregular thoracic aorta (55 mm. in mid-ascending portion) denoting syphilitic aortitis.

It confirmed the diagnosis of aortic syphilis in 17 cases of syphilitic heart disease with aortic insufficiency in which the chest roentgenogram was negative (Figs. 2 and 3), as well as confirming the diagnosis in 4 cases of syphilitic heart disease with aortic insufficiency and 1 case of syphilitic aortitis with aneurysm in which the chest roentgenogram was inconclusive. Seven cases of late syphilis, in addition

to 2 without syphilis, in which aortic syphilis was suggested by chest roentgenogram were excluded from that category by angiocardiography. Aneurysm of the innominate artery was likewise revealed for the first time in 3 patients and ruled out in another originally suspected on chest roentgenogram. Two patients with hypertension and physical findings suggesting possible aortitis, but with negative chest roentgenogram were shown by intravenous contrast study to have minimal smooth dilatation of their aortas commensurate with their hypertension.

In correlating physical findings with results of conventional chest roentgenography and angiocardiography, these have been designated either "positive" or "negative" according to whether or not they indicated a diagnosis of cardiovascular syphilis. Aortic insufficiency and signs commensurate with a large aortic aneurysm were indicative and were considered positive findings. However, the presence of an aortic systolic murmur (and prolonged ringing second aortic sound, if present), considered a positive finding suggestive of syphilitic aortitis, was found to be of little relative or definite value in this group, since it was lacking in 6 of the 16 patients with uncomplicated aortitis and present in approximately three-fifths of the late syphilis patients without cardiovascular syphilis.

Symptoms relative to cardiovascular syphilis as expressed by these patients were of inconstant significance, except in cases of advanced involvement of heart or aorta, so that there has been no attempt to correlate them here.

Summary. Ninety-three patients at the New York Hospital, including 83 with late syphilis, were studied as to the relative merits of physical findings, conventional chest roentgenography, and angiocardiography in the clinical diagnosis of cardiovascular syphilis. By angiocardiography alone, the diagnosis of cardiovascular syphilis was effected in 15, excluded in 9, and sustained in 17 in which chest roentgenography was negative.

Conclusion. Angiocardiography is a valuable instrument in the early diagnosis of syphilitic aortitis. Angiocardiography will also denote adjacent nonvascular masses and reveal unsuspected aneurysms of the aorta and its branches. It is suggested that this procedure may be used to aid in detection of cardiovascular syphilis in patients with late syphilis.

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MICROSCOPIC OBSERVATIONS OF THE CIRCULATING BLOOD OF NINE HEALTHY NORMAL HORSES, ALL OF WHICH HAD UNAGGLUTINATED CIRCULATING BLOOD CELLS AND HIGH IN VITRO ERYTHROCYTE SEDIMENTATION RATES*†

A Contribution to the Theory and General Understanding of the Pathologic Circulatory Physiology of Sludged Blood

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At least since the time of Gulliver[14], 1845, who worked with the blood of healthy cavalry horses, it has been known that the drawn blood of the healthy horse has a high *in vitro* erythrocyte sedimentation rate. In fact, the erythrocyte sedimentation rate of the healthy normal horse is frequently as rapid as that of human beings suffering from rather severe pathologic conditions and diseases. (For reviews of the knowledge of the erythrocyte sedimentation rate in the horse, see Nichols[24,25,26,27,28], 1941 and 1942.

It is sometimes assumed that the results of an *in vitro* erythrocyte sedimentation rate test, done on a sample of a human patient's drawn blood, indicates with more or less precision the degree of intravascular agglutination of the patient's circulating blood. Therefore the purpose of the observations reported in this paper was to find out if healthy normal horses have intravascular agglutination of the circulating blood, that is, "sludged blood." For if the healthy normal horse continuously has sludged blood, how can we believe that sludged blood can or does damage the human body?

The circulating blood of all healthy members of several other species, including human beings, thus far microscopically examined by members of our laboratory, has been completely unagglutinated. *Per contra,* more than 1000 human beings who have had circulating sludged blood have all had clinically diagnosed pathologic conditions and diseases.

In 1947, Knisely, Bloch, Eliot and Warner[16] published a paper in which they outlined 4 major sets of mechanisms whereby they had found sludged blood to damage the bodies of experimental animals and men. These are

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listed, and somewhat elaborated within the paragraphs numbered I, II, III and V below. A fifth new major category of phenomena whereby sludged blood is now known to damage the body is itemized as paragraph IV below. It is here listed as IV to facilitate classification, primarily because some of its items act cumulatively toward initiating factors now listed in paragraph V. The items in IV have previously been known. The accumulating experience of the laboratory now justifies their classification as mechanisms of major importance.

The five currently known major categories are as follows:

I. The resistance of sludged blood to its own passage through the terminal arteriole and capillary bottle-necks of the circulatory system forcibly reduces the rates of blood flow through all the open vessels of the body. This causes various degrees of anoxia (of the stagnant type) of endothelium and tissues, various degrees of loss of fluid from the vascular system through the anoxic endothelium, reduced circulating blood volume, edema of the surrounding tissues, and lastly, it acts toward causing hemocencentration of the blood in the venous system.

II. Agglutinated red cells may be ingested and destroyed by the phagocytic cells of liver and spleen. When it happens, this acts toward reducing both *a*, the red cell count and *b*, the circulating blood volume.

III. There is settling and sedimenting of masses of agglutinated blood cells out of the blood plasma during life, which is a necessary factor in, and may lead to, venous thrombosis. This has now been recorded in motion pictures taken through a horizontal microscope (Warner[33], 1948).

IV. Intravascular agglutination of the circulating blood can operate through other sets of mechanisms both of which plug small vessels: 1, Multiple minute thrombosis, and, 2, Multiple minute embolization.

According to dictionary definitions a thrombus is a mass which plugs a vessel and which was formed at the point where it plugs the vessel. In contrast, an embolus is a mass which plugs a vessel but which was formed somewhere within the vascular system and carried by the flowing blood to the point it plugs, or, in technical language, embolizes.

Following this use of the terminology (which entirely ignores the chemical and, or, more complex or biological factors which may have been operative to form many varieties of either kind of plug) different kinds of sludge have been directly observed with microscopes in experimental animals and sick human beings to cause 1) Multiple minute temporary thrombosis. 2) Multiple minute permanent thrombosis. 3) Multiple minute temporary embolization. This was first recognized as a significant pathologic factor in disease by Bloch[1], 1949, who discovered and studied it in human patients diagnosed as having poliomyelitis. And, 4) Multiple minute permanent embolization.

Minute thrombi are, in our experience, more apt to occur in small veins than in small arteries. Minute emboli are of course much more apt to plug arterioles, capillaries or sinusoids. "Agglutinative thrombi" consisting of masses of agglutinated red cells were reported at least as early as 1902 by Flexner[11]. He saw them in histologic sections in a number of pathologic conditions and diseases, and correctly interpreted their meaning. His paper should be read by all who are interested in sludged blood.

When either multiple minute thrombosis or embolization occurs, the damage done the body acts partly toward impairing the whole circulation and partly toward damaging the particular

organ and tissue in which the plugging occurs. These types of damage are discussed separately as follows:

1. Each time a small vessel is plugged, the flow through that vessel stops. One pathway from the arterial side of the circulation to the venous side is dammed up. The walls of the small vessel, both upstream and downstream from the plug as far as those branches of the vessel which lead to the next adjacent parallel-connected alternative blood pathway, become anoxic and lose their ability to retain blood plasma. Frequently, but not always, the plugged vessel goes through alternate contractions and dilatations, or it may simply relax and become maximally dilated. In many, perhaps most, cases it finally relaxes to its maximal internal diameter. As a direct result of the endothelial anoxia (Landis[22], 1928) the fluid part of the contained blood passes out and through the walls of the vessel and sludged whole blood continues to be forced into the open end of the vessel. Hence, the now maximally dilated small vessel becomes still more solidly impacted with masses of agglutinated blood cells. This differs from simple stasis in that the reduction in blood flow was initiated by the resistance of masses of sludge to passage through the vessel, the vessel is impacted with agglutinated blood (not unagglutinated, separate, but concentrated, blood cells) and such solid impactions are much more difficult to resolve than simple stasis. (Cf. Florey[12], 1926, who described microscopic observations of the resolution of stasis.)

Each time this set of processes occurs, it acts toward reducing the circulating blood volume and venous return, as follows:

a, The stranded masses of blood cells which constitute the minute thrombus or embolus no longer circulate. They are lost from the *circulating* blood. The volume of the *circulating* blood is decreased by an amount equal to the *total* volume of the blood cells in the dilated, impacted vessel. This volume is almost always notably greater than that of the plug or plugs which initiated the impaction.

b, The blood fluid, whatever the composition of the dissolved solutes, is lost, at least temporarily, from the vascular system. By this amount the circulating blood volume is also still further decreased.

c, The impacted masses, whether thrombi or emboli, directly plug one pathway by means of which blood could have passed from the arterial system to the venous system.

The summation of these 3 sets of factors acts directly toward decreasing venous return to the heart.

The exact amount of the decrease in venous return caused at any one time in a subject by these processes obviously depends upon the rates at which these minute thromboses and embolizations and their direct sequelae are occurring, minus the rates at which blood volume restoring processes, and blood pathway reopening processes, are operating.

When but a few minute thromboses and, or, embolizations are occurring, the current clinical methods may detect but little or no changes in the circulation of the living patient.

Enormous numbers of these multiple, minute embolizations and thromboses and their direct immediate consequences occur, both in rapid succession and synchronously *all over the bodies of animals* which (after induction of deep anesthesia) have been caused to develop severe intravascular agglutination of the circulating blood by pounding or burning distinctly localized, limited portions of their bodies (Knisely, Eliot and Bloch[17], 1945; Knisely and Bloch[15], 1945; and Brooks, Dragstedt, Warner and Knisely[3,4], 1948 and 1949). The final

fall of arterial pressure in these 2 conditions does not occur until after great numbers of small vessels, all over the traumatized or burned animals' bodies, are solidly impacted. These and the knowlesi malaria in monkeys currently are the extreme known cases of the operation of this set of pathologic mechanisms upon the whole circulatory system.

2. The histologic and histophysiologic damage done to the whole body by the multiple minute embolization or thrombosis of small vessels in individual organs and tissues depends upon such factors as the following:

a, The particular organ or tissue in which the plugging occurs. Some tissues, such as areolar connective tissues, ligaments, tendons and striated muscles, are composed of histologic elements which can withstand decreases in blood supply or even zero blood supply for considerable periods without suffering (detectable) immediate serious damage. Other organs, such as central nervous system, or the walls of large vessels (O'Neill[29], 1947) consist of elements which withstand deprivation of a blood supply much less well. Some neurones are known to die within 10 minutes after initiation of complete anoxia.

Warner[36], 1949, has taken motion pictures which show masses of sludge plugging individual kidney glomeruli of amphiuma.

(It would be interesting to know whether the diabetic phenomena which sometimes follow infectious disease are caused in part by multiple plugging of the quite individual circulatory systems of great numbers of pancreatic Islands of Langerhans.)

b, The numbers of reserve histological units in the damaged organ. This is what the engineers call "The Factor of Safety". The various organs do not, of course, have identical safety factors.

c, Whether each plugging is permanent or temporary.

d, If temporary, then the *duration* of each period a vessel is plugged is important, and so is

e, The *frequency* with which each vessel is temporarily plugged. The frequency with which each terminal vessel is temporarily embolized depends mostly upon the concentration within the arterial blood of those masses just large and rigid enough to plug the narrow vessel, but still sufficiently small and soft to be distorted slowly and pushed at a later time through the vessel.

f, The rates in time at which the plugging occurs. The total accumulated damage must, of course, be due to the summation of the rates of plugging, minus the summations of the rates of repair and regrowth in the tissues so plugged. It is, of course, well known that different organs vary enormously in their abilities to repair and regenerate.

Currently, it seems increasingly probable that the factors listed in this subdivision of category IV are, when acting somewhat slowly, or intermittently over periods of years, one set of pathologic phenomena causing geriatric changes in the body.

V. Various degrees of reduction in circulating blood volume caused by items I and II and IV above, initiate (probably by way of vasomotor nervous control) intermittent prolonged controlled shutting off of the arterioles and other small vessels of a selected series of tissues and organs. This still further reduces the blood supplied per unit time to certain specific tissues (Knisely, Bloch and Warner[20], 1948).

Rhesus monkeys with knowlesi malaria which go into stage III of the pathologic physiology of that disease, die (if untreated) as a direct result of the above 5 sets of mechanisms, which in that condition are acting to a maxi-

mal and rapidly lethal degree (Knisely, Stratman-Thomas, Eliot and Bloch[18], 1945).

Thus, to return to the major problem of this paper, if healthy normal horses do have circulating agglutinated blood, then 2 (not necessarily mutually exclusive) alternatives must be faced. These are:

1, The horse must have *a*, a vascular system whose arteriole and capillary bottlenecks are either larger or more distensible than those of other species, and, or, *b*, circulatory mechanics differing from those other species, and *c*, mechanisms for the selective phagocytic removal of solid particles from the blood stream by phagocytes of spleen and liver significantly different from the phagocytic mechanisms known to be operative in frogs and rhesus monkeys, or,

2, The concept that sludged blood constitutes one of the major sets of mechanisms whereby pathologic conditions and disease damage the body must be seriously challenged.

If the healthy normal horse, which does have a high in vitro *sedimentation rate, continuously has sludged blood, how can we believe that sludged blood can or does damage the bodies of sick animals and men?*

The background material may be elaborated in more detail as follows:

Knisely, Eliot, Bloch and Warner[16], 1947, reported the specific characteristics of the healthy normal blood and vessel walls of about 3500 healthy frogs, 1100 salamanders, 500 laboratory mammals, and of 50 healthy unanesthetized medical students and student nurses. They reported 1, the circulating red cells were not agglutinated but tended to repel each other slightly. In carefully handled tissues even red cell rouleaux were not present. 2, No white cells or erythrocytes stuck to the inner surfaces of undamaged, healthy, normal, small vessels. 3, The flow of the unagglutinated blood was laminar or streamlined. The cells of the axial stream were arranged in concentric laminae, the center one passing along most rapidly and each additional layer passing along more slowly than the one inside it. One most important observation for this paper was that the wall of each lamina consisted of unagglutinated blood cells; each layer was *exactly* one red cell thick.

The terminal arterioles are long narrow, progressively tapering cones which join capillaries or sinusoids. The narrowest portion of each such terminal arteriole and capillary or sinusoid can contract so tightly shut that nothing can pass through, is under most circumstances so narrow that every red and white cell passing through must be folded or distorted, and when this narrowest portion is maximally relaxed it is only from 1 to 2 or 2½ times the diameter of the animal's own blood cells. (For additional statements of concepts of the internal diameters of arterioles, metarterioles, capillaries, and special capillaries, see Krogh[21], 1929; Clark and Clark[8], 1935; Zweifach[37,38], 1937, 1940; Fulton and Lutz[13], 1942; Chambers and Zweifach[5], 1946; and Knisely, Bloch and Warner[20], 1948).

Arteriovenous anastomoses have been found in some tissues (reviews by Clark[7], 1938; Boyd[2], 1939; and Clara[6], 1939) but these are so few in number and so widely scattered that they cannot nourish the cells of the tissues. If the cells of the tissues and organs are to receive sufficient oxygen and other anabolites and the metabolites be removed effectively, then the blood must flow *through tissue capillaries* and it *must flow rapidly*. Thus, as is well known, the terminal arterioles and capillaries or sinusoids are, under all conditions of health and disease, a perpetual bottle-neck in the vascular system.

Professor Robin Fahraeus[9], 1929, in his Physiological Reviews article entitled "The Suspension Stability of the Blood" mentions on page 261 a microscopic study of the blood stream in the fine vessels of the mesentery of the living horse. He states that "The study of the blood stream in the fine vessels of the mesentery of the living horse does not give at first sight the impression of a granulated stream. But closer observation makes it clear that this is due to the fact that the corpuscles are so strongly aggregated that they do not separate. The blood of the horse in the finer vessels is really not at all a suspension, the erythrocytes running along as a compact coherent string in the middle of the vessel; only when the stream is slowed by pressure on the supplying arteries does the string show granulated outlines and finally break to pieces."

Professor Fahraeus does not state the number of horses studied, nor whether they were carefully selected and cared for, to see that they were healthy before the observations began. He does not state the anesthetic used, the precautions taken to keep the tissues moist and at body temperature, the duration of the experiments before the microscopic observations were made, the magnifications employed, nor whether any precautions were taken to prevent the blood flowing beside the injured body wall in what would seem, in a horse, to be a very extensive laparotomy, from precipitating and agglutinating into a thick pasty sludge as has been described by Knisely, Eliot, and Bloch[17], 1945. Further, he does not state whether the animals observed recovered from the operation.

Also, Knisely and Bloch[15], 1945, and Knisely, Bloch, Eliot and Warner[16], 1947, reported a survey in which now more than 1000 human hospital patients having more than 50 different clinical diagnoses were each found to have intravascular agglutination of the circulating blood. The masses of which these different blood sludges were composed were not rouleaux. The masses were larger and more rigid than the quite familiar serpentine rouleaux. Further, the largest masses were large enough to cause easily visible permanent emboli which plugged terminal human arterioles.

Two general, separate and distinct types of blood cell masses have been recognized in this study.

1. Those which *do not break up into smaller parts* as they are carried into and forced along down through the long progressively narrow, cone-shaped arterioles, terminal arterioles and the narrow, cylindrical capillaries. By arbitrary definition, these masses which do not break up have been named the basic masses of a sludge, usually abbreviated, particularly in conversation, to "the basics".

By means of high speed flash photography each exposure lasting approximately 1/2000 second, the fact that the "basics" are present in arteries has been recorded in colored still pictures (Life magazine[23] of May 31, 1948). Further, the basic masses can be seen in arterioles in the scenes following titles no. 28, 65 and 77 in the motion picture "Knowlesi Malaria in Monkeys". (Knisely, Stratman-Thomas, Eliot and Bloch[18], 1945).

2. Cohering aggregates of basic masses. These aggregates frequently or ordinarily are considerably larger than the basic masses of which they are composed. The aggregates *do* break up into smaller parts as they are forced down through long, cone-shaped arterioles, etc. Each such aggregate breaks up into its own component "*basics*" when it passes through an arteriole and, or, capillary. And, in many patients, the "basics" so released reform in new combinations, into new aggre-

gates as they are carried into small venules in which the blood is flowing slowly.

Plowman[30], 1920, studied, as far as can be determined at this late date, the rates of re-agglutination of basic masses in small retinal veins. As his observations were limited by the very low magnifications and resolving powers of an ophthalmoscope, he certainly did not see terminal arterioles, capillaries or individual basic masses.

These 1000 *in vivo* samples of sludged human blood have been observed microscopically while circulating to vary *a*, from disease to disease, and *b*, in some carefully studied cases during the course of *one* disease in *one* patient. These different sludges varied in such easily observable fundamental factors as the sizes (all comparisons were made using ocular micrometers), statistical distributions of sizes, the degrees of plasticity or rigidity, and of elasticity, toughness, or fragility, and mutual stickiness of their basic masses. (This last may be taken as a direct indication of differences in the strengths of mutually attracting charges on the basic masses.) And the sludged blood in different human patients varied in the internal cohesiveness of their charge aggregates.

The sizes and degrees of internal rigidity of the basic masses are 2 factors which determine the resistance of the basics to their own passage through terminal arterioles, capillaries or sinusoids. In quartz rod transilluminated internal organs of mammals, in which water immersion objectives giving magnifications up to from 400 to 600 diameters can be focused, it is easy to see small arterioles, terminal arterioles, and capillaries forcibly bulged by the basic masses being pushed in single file through them. Sometimes, if the masses are sufficiently large and rigid, each vessel is bulged by each passing mass just as a snake is bulged by a large frog it has swallowed. Further, the masses are somewhat compressed and elongated by the compressing force exerted on them by the walls of the vessels through which they are passing.

The degree of this elongation is, of course, an expression of the degree of internal rigidity or plasticity of the basic mass. The less rigid the mass the more it is squeezed out and the longer it becomes.

When an animal has a homogeneous sludge, that is, when all the passing basic masses are about the same size and have similar degrees of internal rigidity, then the larger and more rigid the basic masses are, the greater each mass bulges the vessels through which it is passing and the slower the blood flows through each arteriole, terminal arteriole, and capillary blood pathway.

One of the next significant problems on which we need to work intensively in this laboratory is the determination of the diameters of the largest basic masses which will go through the terminal arteriole-to-capillary bottle-necks at times when *a*, the arterial and venous pressures are within the normal ranges, *b*, the bottle-necks are maximally dilated, *c*, the basic masses are maximally compressed and *d*, the masses still go through. Preliminary measurements with a not too accurate method indicate that homogeneous *spherical*, rigid, basic agglutinated red cell masses about 30 to 35 micra in diameter are just able to pass through rather widely dilated dog arterioles and capillaries. Many and more accurate measurements of this kind made in different parts of one animal, and in different species of animal, are now obviously needed. For the exact external diameters of compressed masses passing through maximally dilated and bulged vessels constitute limiting cases; all "circulation", hence all vertebrate life, must occur when the basic

masses of homogeneous sludges are smaller than this.

In an animal in which the basic masses are of mixed sizes and degrees of rigidity, some being larger and more rigid than others, the largest and most rigid masses can be seen, under water immersion objectives, to bulge blood vessel bottle-necks and embolize them.

The resistance which sludged blood presents to passage through the arteriole-capillary bottle-neck depends upon 4 factors, 2 of which are properties of the basic masses and 2 properties of the walls of the bottle-necks. These are, *a*, The sizes and degrees of internal rigidity of the masses, and *b*, The internal diameter of the bottle-neck, and the degree of distensibility of the bottle-neck at that particular diameter. The latter factors are usually called the "tonus" of the vessel. The forcible bulging of a vessel by a mass must be an energy consuming process. This energy can come only from the arterial pressure. The elastic return of the vessel behind the forward moving mass may return some of this energy to the forward moving blood. The deformation of a mass from an approximate sphere to an approximate spindle shape must also be an energy consuming process, taking energy which also can come only from the arterial pressure.

Thus it is important to note that the passage of sludged blood through the vascular bottle-necks presents problems in rheology much more complicated than the flow of a simple viscous or Newtonian fluid, or of unagglutinated blood, through narrow, cylindrical tubes with rigid walls.

After one has had rather extensive experience studying the passage of agglutinated masses through vessels at high magnification in animals, it becomes possible from time to time under favorable conditions to observe the same kinds of phenomena at lower magnifications in the bulbar conjunctival vessels of sick human hospital patients.

A detailed account of the known mechanisms whereby sludged blood damages the body has already been mentioned. Further, a detailed account of one mammalian disease in which the mechanisms of sludge are the major cause of death has also been published, documented with motion pictures taken through the microscope (Knisely, Stratman-Thomas, Eliot and Bloch[18], 1945).

In a review article in the Swedish language, entitled "Den Intravasala Erythrocytaggregationen, dess Historiska och Fysiologiska Betydelse" (translated) "The Intravascular Erythrocyte Aggregation, Its Historical and Physiological Importance", Professor Fahraeus[10], 1948, again pointed out "that microscopic studies of the flowing blood of the paracapillary vessels of the horse have convinced me that in this case the blood can hardly be called a suspension." He does not say that he has made additional microscopic observations of circulating horse blood since 1929. Toward the end of the paper, Fahraeus says "as already stated, the mentioned characteristics of horse blood are in favor of the view that a very low suspension stability can be quite a normal phenomenon, which constitutes a warning against drawing impulsive conclusions concerning the pathological significance of 'sludged blood'."

Thus, we are faced with the problem of whether or not healthy normal horses have intravascular agglutination of the circulating blood. What is special about horses' blood? If the blood of healthy horses is agglutinated *in vivo*, are the masses hard, or soft so that they may easily be squeezed out into long plastic spindle-shaped masses as they are forced to pass through terminal arterioles into capillaries? Or, is it possible perhaps that the terminal arteri-

oles and capillaries of healthy normal horses have considerably wider diameters than those of other species of animal? Or are they more easily distensible?

Further, what special features of circulatory mechanics prevent masses of agglutinated horse red cells from settling by ordinary gravity to the lower sides of horizontal or nearly horizontal vessels? (Warner[35], 1948.)

There is yet another aspect of this problem: Knisely, Bloch and Warner[20], 1948, have observed the circulation in the sinusoids of living transilluminated livers of frogs and rhesus monkeys. In each of these species the sinusoids are branching and anastomosing cylindrical tubes, each completely lined with cells which are highly efficient at removing certain types of foreign particles from the circulating blood. In the frog, minute particles of India ink, kaolin and graphite injected into the vascular system, have been directly observed to receive a coating of glassy material, which completely covered each of these particles. Whenever such coatings, containing particles of the above materials, touched the sinusoid linings of the liver, each coating with whatever it contained was instantly selectively phagocytized from moving blood.

In rhesus monkeys during Stage II of knowlesi malaria, each parasitized red cell receives a clear glassy coating of a precipitate which completely surrounds the parasitized red cell (Knisely, Stratman-Thomas, Eliot and Bloch[18], 1945; and Knisely, Bloch, Eliot and Warner[16], 1947). These coatings have been photographed by dark field *in vitro* (Reel II of the Knowlesi Malaria film). The coatings also act as opsonins by sticking the parasitized red cells to the hepatic sinusoid lining cells which rapidly ingest the coatings with, by necessity, the contained parasitized red cells.

Following burns in dogs (Brooks, Dragstedt, Warner and Knisely[3,4], 1948 and 1949) coatings form on groups of red cells and stick them together in hard clumps. These coatings also almost certainly act as opsonins, for clumps of such red cells have been found in the phagocytes in histologic sections of spleens and livers of these burned dogs.

In both frogs and rhesus monkeys, normal, naked, uncoated, unagglutinated red cells have been watched sliding and bumping along the inner surfaces of the highly phagocytic, hepatic, sinusoid lining cells and not one single naked unagglutinated red blood cell has ever been observed to be selectively ingested.

Thus, we are faced with the following additional problems: If the healthy normal horse does have intravascular agglutination of the circulating blood, then we must seek a whole new series of answers about the selective phagocytic removal of particles from the blood by the phagocytes of horse spleen and liver.

a, Perhaps agglutinated horse red cells are not coated, or are not phagocytizable.

b, Does the horse perhaps have special mechanisms to prevent the selective ingestion of its own agglutinated blood cells?

c, Does the horse perhaps continuously have a very high rate of phagocytic destruction of agglutinated blood cells and a correspondingly high rate of erythropoiesis?

Materials and Methods. Nine healthy normal horses were selected for microscopic study by members of the faculty of the College of Veterinary Medicine of the University of Illinois at Urbana. It is important to note that these horses were selected by accredited veterinarians. Further, the horses selected had been under the routine care of these veterinarians, a part of whose responsibility it is to maintain the livestock of the agricultural school at the highest degree of good health.

One horse was a dappled gray mare, weighing approximately 1800 pounds. She was an exceptionally healthy animal, kept by the veterinary school for one purpose only: that of supplying blood with which to make serums. She was trained to be led with a halter, but was not trained to wear a harness or a saddle, and had never done any physical work of any kind. Various members of the veterinary staff volunteered information that they had never seen her exhibit any sign of illness during the last consecutive 6 years.

This horse was particularly valuable for microscopic study of the circulation, not only because of her past record of good health, but also because the veterinary school no longer needed her to donate blood (because one other similar horse was sufficient for that), could not sell her because she was not trained to work and, therefore, intended to kill her to use the horse meat to feed the members of their dog colony. As will be noted later, the circulating blood of this healthy horse was studied carefully 2 different times, a week apart, the horse was then killed and autopsied carefully, which provided a final control on the previous good health of this one animal.

The other 8 horses consisted of 4 two-horse teams which routinely were doing light work on the farms of the agricultural college.

The veterinary school has a new surgical operating table for large animals. It was not quite complete; hence, it was necessary to improvise some temporary arrangements for holding the horses still, particularly the equipment for binding the horses feet had to be improvised. For binding, large-diameter soft cotton (not sisal) ropes were used. They were approximately 1 inch in diameter, were quite new and soft, and were never bound to the horse more tightly than was necessitated by the degree of kicking and jumping which the animal did at a given time. Knisely, Eliot and Bloch[17], 1945, have shown, with motion picture records taken through the microscope, that healthy blood flowing in or near mechanically traumatized tissues changes from its unagglutinated state to heavily precipitated and agglutinated blood. This was why the horses were treated as gently as possible, the binding ropes soft, and not tied more tightly than was necessary.

The steel operating table could be swung into the vertical plane, a horse led up beside it, and bound loosely to this plane, which seemed as solid as the wall of a building. After the horse was loosely attached, an electric motor acting through a hydraulic pump, smoothly pulled the hinged vertical table into the horizontal plane, the final result being that the horse was lying on a table approximately 30 inches high. The various flat straps around the horse's belly and ropes around ankles were then pulled a little tighter, some heavy cloth was put under the horse's head and the head then tied down with flat straps and soft cords. Several attendants, men known to the horses, stood ready to help quiet the horse if it should begin to thrash about.

Then a bi-objective, binocular, stereoscopic Leitz microscope of the type shown in Fig. 1 of Knisely, Bloch, Eliot and Warner[16], 1947, was focused onto the horse's bulbar conjunctiva and, or, nictitating membrane. These tissues were illuminated obliquely by means of a Shahan's ophthalmoscopic lamp.

The method permits direct microscopic study of one set of local blood vessels together with a statistically valid sample of all the animal's circulating arterial blood. The fact that this is a statistically valid sample of the circulating arterial blood of an animal has been checked in rhesus monkeys, dogs, cats, rats, guinea-pigs and mice by opening an individual animal widely and studying the mechanical condition of the blood in tissue after tissue. It was first checked in a rhesus monkey with knowlesi malaria. In this monkey observations were made on the surface of the exposed pia arachnoid, surfaces of mesenteries, omentum, outer surfaces of many areas of small and large intestines, edge of the liver, ureters, small vessels within large nerves, pancreas and some easily available inner surfaces of the striated body wall musculature, together with observations of blood flowing on the inner surface of the everted eyelid and of blood flowing through small vessels in the bulbar conjunctiva. In all these places, the mechanical condition of the blood coming down the arterioles was identical. Similar observations have been made in traumatized dogs, other laboratory animals also traumatized, burned dogs and in great numbers of animals each of which was opened with the previously described (Knisely, Eliot and Bloch[17], 1945), bloodless, sludgeless operation. From this type of experiment we know that the blood coming down any set of arterioles in the body is a statistically valid sample of the blood coming down all other arterioles of the body. This makes it possible to study the blood coming down the arterioles of the bulbar conjunctiva and nictitating membrane of experimental animals and be certain that we are seeing a valid sample of all of the animal's circulating arterial blood.

This concept does not hold for small veins, for the simple reason that as various types of circulatory pathology become progressively worse, various sets of veins of the body respond at either different rates or to different degrees. Some may plug up with masses of sludge before others, and the degree of charge agglutination in some tissues is greater than in others. "Charge agglutination" was defined by Knisely, Bloch, Eliot and Warner[16], 1947, as the agglutination of basic masses, a set of processes whereby basic masses which do not break up as they are pushed through terminal arterioles and capillaries undergo a loose, secondary agglutination as they pass through small veins. This agglutination in small veins has been observed by many previous investigators, most of whom were not too certain of exactly what could be seen with any given degree of magnification of the microscopes they employed.

In summary, microscopic observation of the nictitating membrane or bulbar conjunctiva permits study of a set of small vessels and of a statistically valid sample of circulating arterial blood, but does not necessarily give microscopic images of a statistically valid sample of the venous blood of the body.

Results. The first horse to be observed by this method was the 1800-pound dappled gray mare. She was completely unanesthetized for the 1st series of observations. This horse had a large area of bulbar conjunctiva which could be studied easily whenever she would hold her eyes still. When first on the table she rolled her eyes a good deal which made observation impossible. In about 5 minutes, however, during which the horsemen spoke quietly and reassuringly to her, she voluntarily quieted down and became quite relaxed, which made it very easy to study the blood in her bulbar conjunctival vessels. She was on the operating table most of one afternoon during which we had many excellent periods of study and could study both the vessels and the circulating blood at magnifications up to X 64. The stereoscopic microscope in use gave splendid detail at this magnification. Every factor of normal, circulating blood was exactly as we have found and described it in previous studies made in laboratory animals and in healthy, unanesthetized, unoperated human beings: namely

1. The erythrocytes were unagglutinated.

2. Leukocytes were not sticking to the insides of the walls of small vessels.

3. The flowing of the unagglutinated blood was laminar.

4. Small vessels were not leaking fluid rapidly enough to cause microscopically detectable hemoconcentration of the passing blood or edema of the surrounding tissues.

5. The flow in most vessels, which were 60 to 100 micra in diameter, was so rapid that individual red cells could not be seen. In narrower vessels individual red cells could barely be seen at the X 64 magnification. There were spaces between them. Also, it was easy to see that these red cells were not agglutinated, by the simple process of measuring the width of the narrow column of blood cells passing through the smallest vessels. This could be done with an ocular micrometer scale and it showed that the red cells were passing in single file.

Another observational technique, not previously described, also showed that the red cells were not agglutinated. This consists of measuring the thickness of the wall of the individual lamina in the laminar systems of smallest post-capillary venules. (For descriptions of the laminar systems, see Poiseulle[31], 1841, and Thoma[33,34], 1896 and 1927.) In every microscopic field observed, the wall of each of these concentric laminae was exactly 1 red cell in thickness. This can easily be observed in flowing blood and is indisputable evidence that the moving red cells were not held together in clumps.

This was the condition, then, in the early part of the afternoon while this healthy horse was being studied.

The horse held still enough so that many people had a look at her circulation—not only persons skilled at studying circulating blood with microscopes but at least 6 veterinarians who had had no previous experience in making microscopic studies of flowing blood. These men could observe small veins, but it takes more experience to see small arterioles and still narrower vessels. Each of these volunteered that he saw no signs of gaps in the streams of red in small veins and that he saw no signs of "beads" in the small veins, which is evidence that "charge agglutination" was not occurring in these veins. (These veterinarians had seen the motion picture, "Knowlesi Malaria in Monkeys," hence were conscious of the fact that separate masses might be observable in veins of living animals.)

Dr. Gregg, veterinarian, drew a sample of blood from this horse's jugular vein and ran a sedimentation rate test upon it. The blood cells sedimented very rapidly. In a column of blood 5½ inches high in a 6-inch test-tube, the upper surface of the suspended red cells fell ⅛ inch, (approximately 3 mm.) in the first 5 minutes, and 1⅛ inches (approximately 28 mm.) in the first 20 minutes. The veterinarians who were watching the work said that the cells of this blood settled as rapidly as is usually found in apparently healthy horses.

These observations permit the first major deduction, which is that the rapid sedimentation rate of the blood of healthy horses *in vitro* cannot be taken as evidence that the horse has intravascular agglutination of the circulating blood. As a direct first corollary, it is necessary to conclude that some alteration in the mechanical characteristics of the blood occurred either *a*, while it is being drawn out of the horse, or *b*, almost immediately after it is put into the sedimentation rate tube.

This horse was anesthetized with chloral hydrate and the animal's blood circulating in small vessels observed again. Following the chloral hydrate anesthesia, the animal did not have or develop agglutinated blood; hence, it is possible to conclude that chloral hydrate does not always cause intravascular agglutination of the blood and may never cause intravascular agglutination of the blood.

A number of non-scientific attendants were watching this whole procedure, one of whom accidentally performed the following maneuver. He did not know the delicacy of living tissues and without warning to us, reached a finger onto the anesthetized horse's bulbar conjunctiva, his purpose being to pull the nictitating membrane out over the surface of the eye to make that tissue more easily observable. His finger slipped and he pushed the finger hard against the side of the horse's bulbar conjunctiva. Had the horse not been anesthetized, the finger jab against the side of the eye would have been quite painful.

Immediately after this moderate degree of trauma, we focused a microscope onto this partly injured tissue. All the blood flowing through the small vessels in this injured area changed from healthy unagglutinated blood as it entered, into thick, pasty agglutinated blood as it passed through the injured area or tissue and on into small veins, exactly as in traumatized tissues of other animals.

The precipitation and agglutination of blood flowing through partly crushed tissues has been described in detail by Knisely, Eliot and Bloch[17], 1945. Subsequent unpublished experiments have shown that when a dog is struck from 20 to 40 blows on the back legs, enough sludge comes from this series of multiple injuries to change all the animal's circulating blood to a thick, pasty sludge and that this thick sludge

is a major factor in the particular type of shock which follows mechanical trauma. The precipitation of the blood flowing through crushed tissue has been recorded in colored motion pictures.

Fahraeus[10], 1948, has offered another (and to us unacceptable) explanation for the appearance of masses in blood flowing through or beside crushed tissues. He says, (translated) "I further believe that Knisely is wrong in assuming that erythrocyte aggregation which can be observed in the vessels after trauma is due to a change in the blood called forth by trauma. The explanation is certainly that the mechanical irritation causes vessel contractions which lead to a reduction of blood flow and this in its turn creates the conditions for the appearance of granular flow."

A factor, necessary for this concept to be true, would be the contraction of the vessel in which the sludge is forming, *before* the sludge begins to form.

Since Knisely, Eliot and Bloch[17], 1945, published descriptions of observations of precipitation and agglutination of blood flowing through crushed tissue, the following relevant observations and experiments have been made:

1. The quartz-rod transilluminated edges of laparotomy wounds have been studied with microscopes. Stereoscopic, bi-objective Leitz microscopes, giving magnifications of X32, 48, 64 and 96 have usually been used. Frequent observations have also been made with higher magnification monobjective microscopes in the thinner parts of the edges of laparotomy wounds. The edges of such wounds have been studied in mice, rats, cats, guinea pigs, dogs and rhesus monkeys. In every case wherever blood flowed through or near traumatized tissue, the blood flowing past changed as it flowed from unagglutinated, freely-flowing blood, to thick, pasty, slowly-flowing sludged blood. The masses formed in post-capillary venules and small veins, and remained discrete, solid masses as they were carried into the next larger sized veins and mixed with unagglutinated blood from adjacent untraumatized areas. This occurred whether the vessels in the traumatized tissue were constricted or dilated, and it occurred continuously during periods when such vessels were going through slow alternate contractions and dilatations. Thus, constriction of the vessel is not necessary for initiation of the formation of sludge.

The masses of sludge so formed were not rouleaux, though as noted by Knisely, Eliot and Bloch[17], 1945, short rouleaux were frequently contained within them. The members of our laboratory are, through years of experience, thoroughly familiar with rouleaux. The masses formed in blood flowing through or near crushed tissues are much larger in diameter than rouleaux. The red cells are arranged at all angles to each other within the masses, and the masses are more rigid and are tougher than the ordinary, quite familiar flexible serpentine rouleaux.

There can be no doubt but that many people have, through failure of precise direct observation, and easy uncritical assumption, been calling any set of masses seen in circulating blood, "rouleaux". This, now historic mistake, cannot be permitted to go on, for it has been blinding many to the pathologic importance of the phenomena now under discussion.

2. To find out if substances can come from crushed tissues, pass in through vessel walls, and initiate precipitation and agglutination of the passing blood, the following tests have been made. They consist essentially of taking pulped tissue from a donor animal and placing it upon the surfaces of untraumatized tissue of a test animal. The test is read by means of a microscope focused into untraumatized vessels thus exposed to possible substances diffusing from pulped organs of the donor. In detail, the tests were carried out as follows:

First, a mouse was prepared using a bloodless, sludgeless laparotomy as described by Knisely, Eliot and Bloch[17], 1945. A loop of the animal's small intestine was drawn out so that it could be transilluminated for microscopic study. The mouse was used for 2 reasons: first, the small intestine is so thin that the whole organ can be transilluminated from one side to the other; thus it is not necessary to traumatize mouse small intestine in any way to prepare it for the following microscopic study and tests. Second, the small vessels which nourish the outer layers of the smooth muscle of the intestine are very close to the inner surface of the mesothelium which covers the intestine. Thus, it is an extremely short distance from the outer surface of the mesothelium in through the intestinal wall to the endothelium of the vessels which nourish the gut muscle. To make the test technically simple and thus more easily successful, this distance must be kept as short as possible. Hence a small animal, mouse, was first used. From now on the mouse prepared as outlined will be called the "test mouse," or "test animal."

A careful microscopic study of the exposed

loop of intestine showed that all the blood flowing through its smallest vessels was completely unagglutinated. This could be seen with the stereoscopic bi-objective microscope. Further, we have a regular mono-objective compound microscope mounted on a swing arm which makes it possible to focus high magnification lenses on transilluminated tissues. Magnifications up to X400 showed clearly that all passing red cells were completely free from each other.

Next, another mouse was killed by cutting its head off with scissors, and parts of each of a series of individual organs removed, each by itself, and crushed. This mouse was the donor animal.

A bit of striated muscle from the donor mouse was crushed repeatedly between 2 glass slides, and a droplet of the muscle pulp, about ¼ the diameter of the head of a common pin, placed gently on the outer surface of the transilluminated intestine of the test mouse. While this was done, the test area was continuously under observation. The outer surface of the intestine was not itself touched at all with the instrument which placed the droplet of crushed, striated muscle on the intestine. A small droplet of muscle pulp was used so that it would be possible to focus through its edges and study possible reactions occurring in small vessels within the surface of the underlying small intestine.

Within 2 seconds or less, after the wet, pulped, striated muscle touched the intestine, all the blood passing through the smallest vessels underlying the muscle pulp began to change to a thick, pasty sludge. The sludge was formed in capillaries and small post-capillary venules regardless of the diameters which these vessels had at the moment. Sludge was formed in contracted vessels and in dilated vessels, and it was formed continuously in vessels which were undergoing rhythmic contractions and dilatations. These vessels contracted at times to zero and at times dilated, or relaxed, to their maximal internal diameter. Whatever the diameter of these vessels, the blood passing through them changed to a thick, pasty sludge.

In contrast, in similar vessels of adjacent areas of the intestine which had not been exposed to juices from the striated muscle pulp, no precipitation or agglutination of the passing blood was occurring. No precipitation or agglutination occurred in vessels, whether they were contracted narrowly or relaxed maximally or undergoing rhythmical contractions and relaxations.

Thus, 1. contraction of small vessels did not in itself initiate sludge formation, and 2. in areas exposed to substances coming from the pulped, striated muscle, sludge was formed regardless of the diameters of the small vessels. Thus, contraction of a vessel is neither sufficient, nor necessary, to initiate sludge formation.

The major conclusion from this type of experiment is, of course, as follows: From fresh, mechanically pulped striated muscle, some one or more substances are released capable of *1*, passing, probably by diffusion, through *a*, the mesothelium of the peritoneum, *b*, a thin layer of smooth muscle, and *c*, the endothelial walls of small vessels; and *2*, initiating precipitation and agglutination of the contained passing blood.

From now on, such substances will be called "sludge initiator substances".

Similar tests, using pulped tissues from donor mice have shown that sludge initiator substances come from pulped brain, liver, spleen, smooth muscle taken from the gut wall, testis and kidney. Other tissues have not yet been tested.

Because these particular sludge initiator substances diffuse so rapidly through several thin membranes, including endothelium, it seems quite probable that they have low molecular weights.

Somewhat similar but ultimately more useful tests have been made, using dogs, as follows:

A test dog was anesthetized with phenobarbital. Microscopic observation of the blood flowing through his bulbar conjunctiva showed his blood to be completely unagglutinated (Knisely, Bloch and Warner[19], 1946). He was then opened with a nearly bloodless, sludgeless mid-line laparotomy incision. Immediately afterward there were but small amounts of softly agglutinated blood cells passing through his bulbar conjunctival vessels. Most passing blood cells were unagglutinated.

A donor dog was killed, large pieces of striated muscle taken from his back legs and reduced to a fine wet pulp in a Latine machine. Great double handfuls of this freshly pupled muscle were then placed on a table top right beside the belly of the test dog.

The test dog lay on his side, belly toward the mounds of wet muscle pulp. Next, loops of the test dog's small intestine were gently withdrawn and spread, mesentery and all,

upon and across the wet, finely pulped striated muscle.

A Shahan lamp illuminated the test dog's mesentery from above, and by the reflected light the following could be seen stereoscopically at X32, 64, and 96 magnifications:

1. The blood passing down afferent or arterial branches of the mesenteric vessels was almost completely unagglutinated; it was closely similar to that previously seen in the dog's conjunctiva.

2. The blood returning through the great numbers of venous arcades of the intestinal mesentery was precipitated and agglutinated into great thick, sticky wads and masses.

From the time the donor dog is killed until the test is read need not be more than 10 minutes; there is not sufficient time for bacterial growth to occur in the pulped muscle. Hence, there can be no doubt but that the changes in the mechanical consistency of this blood in the test dog were called forth by chemicals released from the fresh, finely pulped, wet, striated muscle of the donor dog.*

This technique, and modifications of it, are now used in our laboratory to initiate the responses of the blood and circulatory system to the products of mechanical trauma without at the same time stimulating the system by much or any initial loss of blood. Thus, this technique makes it possible to initiate traumatic shock in the absence of hemorrhage.

Knisely, Bloch, Eliot and Warner[16], 1947, described a simple method of initiating, in human blood donors, hemorrhage with, but at most, infinitesimal trauma.

Thus, it is now possible experimentally to initiate hemorrhagic shock without trauma, and traumatic shock without hemorrhage.

To return to the problems of horse blood, the fact that the blood of the healthy 1800-pound dappled gray mare changed to a sludge as it passed through her own crushed tissue, sharply re-emphasizes the necessity for making all microscopic studies of the blood of healthy horses under conditions which insure that no mechanical trauma to the horse takes place, for trauma would initiate the formation of sludge within the horse's circulatory system.

The formation of sludge in this anesthetized horse following mechanical trauma also showed that chloral hydrate did not prevent the formation of sludge in a traumatized horse. Further, one may summarize and say that chloral hydrate does not, in itself, initiate a sludge in a healthy horse nor prevent trauma from initiating sludge in a healthy horse.

This horse was observed again under similar circumstances one week later and found to have unagglutinated blood and healthy vessel walls exactly as previously described.

The horse was then killed and autopsied. A few parasitic worms were found in the intestine, several different species being present, all of which are rather standard parasites of horses in that part of the country. Other than these worms, a gross autopsy, carefully done by skilled practicing members of the Department of Veterinary Pathology, disclosed no detectable pathological conditions within the animal. This was the most carefully done experiment on a healthy horse.

From this experiment it is possible to conclude:

1. That one healthy horse had completely unagglutinated blood cells as previously described for healthy specimens of other species.

2. Chloral hydrate does not itself necessarily initiate agglutination of circulating horse blood.

3. Chloral hydrate did not prevent trauma from initiating agglutination of

* These tests, which showed that substances from pulped, striated muscles of dogs can diffuse in through the walls of dog mesenteric and intestinal vessels and initiate precipitation and agglutination of the passing intravascular blood, were performed in the Department of Physiology of the University of Tennessee Medical School at Memphis in the spring of 1942 by M. H. Knisely, S. Barker, E. H. Bloch, A. Lipscombe, and L. Warner. Extensive descriptions of experiments on traumatic shock based on the above observations will be published in detail later.

the blood passing the traumatized territory.

4. It is necessary to keep horses protected from trauma if the unagglutinated blood of healthy horses is to be seen by microscopic study.

5. The sedimentation rate test performed immediately upon freshly drawn, previously unagglutinated blood of a healthy horse exhibits a high sedimentation rate, and

6. It is necessary to conclude that the high rate of sedimentation of the red cells of blood of a healthy horse does not necessarily indicate that the horse had intravascular agglutination of the circulating blood.

Each of the 8 other horses, with but 1 exception, were prepared for observation exactly as outlined above. The exception provided some additional knowledge about the responses of horses to mechanical trauma; hence, it is here described in some detail.

Because the whole procedure of tying the horse on an operating table is somewhat laborious and time-consuming, serious effort was made to see if we could fasten a microscope on a swing arm to a tall, vertical post in the yard, lead a horse close to the quite maneuverable microscope and study the horse's bulbar conjunctival vessels and blood while the horse was standing up. The first trial was a complete failure because the horse was afraid of all the equipment which was brought within a short distance of his eye. An attendant suggested holding the horse's head still by means of what horsemen call a "twitch", a loop of small rope which is put around the horse's upper lip and which can be pulled very tight. The twitch is a painful instrument and in most circumstances, once it is on a horse, the horse will hold quite still. A twitch was put on this horse and tightened, and, seen grossly, the horse stood still. But, whenever we tried to shine a light on the side of the eye and focus a microscope, he made small dodging motions to avoid the light so that it was never possible to get a microscope focused on the eye of this horse while he was standing up.

The series of small dodging motions was interrupted at times by hard, quick jerks made by the horse. He would throw his head backward which made a sudden, hard pull on the rope around his upper lip. Finally, this attempt was given up and the horse taken in and put on the operating table. When the microscope was focused on his bulbar conjunctiva, almost all his blood was found to be unagglutinated, but from time to time small discrete masses of agglutinated red cells came down through the arterioles of the nictitating membrane and bulbar conjunctiva. These masses were scattered at intervals among the unagglutinated red cells. In unpublished experiments we have focused a microscope on the side of the eye of an anesthetized dog and then struck the back leg of the dog a number of hard blows with a hammer. In each case, after from 20 to 40 seconds, small masses of red cells tightly stuck together, could be seen coming down the arterioles of the dog's nictitating membrane and conjunctiva. The greater the number of blows struck the dog and the longer time afterward, the greater the *concentration* of small masses coming down the arterioles of the membrane and bulbar conjunctiva. Thus, the appearance of a few scattered small masses coming down the arterioles of this horse, whose upper lip had been traumatized for approximately a half-hour, is in strict agreement with the dog experiments. Following trauma, some of the masses produced at the site of the lesion are tough enough to maintain themselves as solid masses as they are carried around and around through the whole circulatory system.

In summation, in this horse almost

all the red cells were unagglutinated. The scattered masses of agglutinated cells almost certainly came from the upper lip which was known to have received pretty rough treatment during the immediately previous half-hour.

The other 7 horses had completely unagglutinated blood and normal vessel walls exactly as has been described for other species and for the 1800-pound mare.

Blood from all 9 horses showed the high sedimentation rates known to be common to all healthy horses. Hence, from this group of experiments it is possible to conclude that:

1. The circulating blood of healthy horses is not agglutinated.

2. It is necessary to keep horses protected from trauma if the unagglutinated blood of living healthy horses is to be seen by microscopic study.

3. The sedimentation rate test performed immediately upon freshly drawn, unagglutinated blood of healthy horses exhibits a high sedimentation rate.

4. It is necessary to conclude that the high rate of sedimentation of red cells of the blood of healthy horses does not necessarily indicate that the horse had intravascular agglutination of the circulating blood.

5. It is necessary to conclude that some alteration of the mechanical characteristics of the blood of healthy horses occurs *a*, while it is being drawn out of the horse, or, *b*, immediately after it has been put into sedimentation rate tubes (or both), in order to produce the high *in vitro* sedimentation rates characteristic of the blood of healthy horses.

Discussion. It seems quite probable that the intravascular agglutination of circulating horse blood seen by Professor Fahraeus in exposed horse omentum was caused, at least in part, by the trauma done to the horse's tissues by the surgery involved in making the laparotomy. As noted, Knisely, Eliot and Bloch[17], 1945, found that the blood flowing through vessels beside crushed tissues changes from its normally unagglutinated, relatively fluid state to a precipitated and agglutinated sludge. Since then it has also been found in our laboratory that if the surfaces of abdominal organs, such as intestines, mesenteries or omentum are permitted to lie across the raw surface of the cut body wall, the blood flowing through the enormous numbers of small vessels just beneath the surfaces of the intact abdominal structures similarly changes to a sludge which is poured into the general circulation. Materials diffuse from the cut body wall musculature in through the peritoneal surfaces and small vessel walls of directly adjacent intestine, mesenteries or omentum and initiate the precipitation and agglutination of the passing intravascular blood. In our laboratory, great care is now being taken to be sure that internal abdominal structures do not drag against cut surfaces of body wall during our surgical procedures.

For these reasons, it seems necessary to assert that the microscopic observations which Professor Fahraeus made previous to 1929 in mesenteric vessels of the laparotomized horse are not necessarily relevant to the current discussion.

As the blood of these 9 healthy horses was unagglutinated, there is now no reason to assume that circulating healthy horse blood resists its own passage through bottle-neck vessels more than does the unagglutinated blood of other species. Further, as the healthy horse blood was unagglutinated, there is no reason to suspect that *healthy* horse red cells are in a condition ready to be selectively phagocytized from the passing blood by the phagocytes of the spleen, bone marrow and liver.

These 2 factors are particularly im-

portant, for they begin to lay the foundation for understanding the circulatory physiology of healthy horses, in preparation for study of the pathologic physiology of horses suffering from various pathologic conditions and diseases. It is particularly important as a preparation for studying horses suffering from "Infectious Anemia of Horses" (Stein[32], 1942) which recently caused serious damage to racing stock in this country.

Further, and for the moment rather important, the high *in vitro* sedimentation rate of the healthy horse cannot be taken as evidence that healthy horses have circulating sludged blood, nor can the high *in vitro* sedimentation rate of blood from healthy horses be used as evidence with which to minimize the importance of the damage done to living human patients by sludged blood.

In spite of the disagreements with Professor Fahraeus on the above few points, it is a privilege to note that many of the experimental results from our laboratory are in strict agreement with, and a direct extension of many of the concepts of how agglutinated blood might damage the body, published by Professor Fahraeus in the latter part of his article in 1929[9]. In fact, for the sake of historical perspective, it is worth while to note that our microscopic studies in living experimental animals and in sick human beings are a direct experimental continuation of the studies of agglutinated blood which, in our generation, were initiated by him.

Summary. 1. Nine carefully selected healthy horses have had unagglutinated circulating blood cells.

2. Blood from each has shown the high *in vitro* sedimentation rate, well known to be common to samples of horse's blood.

3. Trauma to 1 healthy horse caused microscopically observed precipitation and agglutination of blood passing through vessels in and near the sites of the trauma.

4. Trauma to 1 other horse caused precipitation and agglutination of masses, many of which were sufficiently hard and rigid to remain intact while being carried around and around within the horse's circulatory system.

5. It is necessary to keep horses protected from mechanical trauma, if the unagglutinated blood of living healthy horses is to be seen by microscopic study.

6. The high *in vitro* sedimentation rate of blood from healthy horses cannot be used as evidence with which to minimize the importance of the damage done to living human patients by sludged blood.

The authors wish to express deep appreciation to Drs. R. P. Link, A. L. Gregg, H. Hardenbrook, Jr., and S. C. Schmittle, who carefully selected the healthy horses and managed the difficult process of tying them down for microscopic observations; to Dr. C. C. Morrill, Professor of Veterinary Pathology, who performed the autopsy; and to Professors R. Graham, Lloyd E. Boley, and R. P. Link whose courtesy and administrative care made this study possible.

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CONGENITAL MITRAL ATRESIA

Report of Two Cases

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Recent developments in the surgical treatment of congenital cardiovascular disease require accurate distinction between operable and inoperable malformations. The recording of rare maladies of this type, even though inoperable, is, therefore, justified. Of the cardiovascular anomalies, atrio-ventricular septal shift-to-the-left, with or without mitral atresia, is among the rarest. We have found 32 cases in the literature to which we would like to add 2 recent observations. Harris and Farber[7], in their critical review and case presentation, accepted 25 cases, including their own. They discussed the phylogenetic principles of its development on the basis of Spitzer's theory, as well as the development of associated anomalies, with special emphasis on the concomitantly occurring aortic dextroposition. Subsequently, Gibson and Clifton[6], Walls[19], Krumbhaar[9], Walker and Klinck[18], and Manhoff and Howe[10] have presented reports of single instances of this disorder. Finally, Taussig reported 2 cases[16,17]. We believe ours to be the 33d and 34th reported cases.

Case Reports. Case No. 1. *Clinical Summary.* A 14-day old white male infant was born following a normal gestation and delivery. The mother had given birth to a stillborn infant 2 years previously, but otherwise the familial history was not remarkable. The family physician stated that the infant appeared to be slightly cyanotic at birth, and throughout its life. A cardiac murmur or enlargement was not detected. During its second week of life, the baby was at home, and according to the parents appeared normal, slept, took its formula well, and was never cyanotic until 30 minutes prior to admission to the Charlotte Memorial Hospital. While nursing, the baby suddenly gave a cry, developed difficulties in respiration, broke out in a cold sweat, and became markedly cyanotic. It was brought immediately to the hospital, where oxygen, coramine, adrenalin, and finally artificial respiration were virtually non-effective. It expired ¾ of an hour after the inception of the acute attack described.

Autopsy Findings. The body was that of a well-developed white male infant, measuring 37 cm. in length (crown-rump), and weighing 3845 gm. The pertinent findings were limited to the heart and the great vessels.

The pericardial sac measured 6.5 cm. in its greatest transverse diameter, and contained no excess fluid. The parietal and visceral pericardium were smooth, moist and glistening.

The heart (See Figs. 1 and 2) weighed 42 gm., the normal weight at 2 weeks of age being 19 gm.[2]. It occupied a central thoracic position, and was globular in shape with a well rounded apex. The venae cavae terminated normally in an extraordinarily dilated right atrium, which was about 4 times its normal size, and the myocardium of which measured 0.2 cm. in thickness. The orifice of the coronary sinus was somewhat dilated. The right atrio-ventricular valve measured 5.8 cm. in circumference, and its leaflets were attached by rather widely separated chordae tendineae to markedly hypertrophied ventricular papillary muscles, the largest of which measured 0.6 cm. in thickness. The right ventricle was extremely dilated, and its cavity constituted all of the heart's functioning ventricular volume. The right ventricular myocardium measured 0.6 cm. in thickness, and was very flabby in consistency. The myocardium was normal. The ostium of the pulmonary artery was situated between the tricuspid valve and the aortic ostium, and was guarded by 3 well-developed semilunar cusps. The pulmonary valve and first portion of the pulmonary artery were normal in circumference. The vessel gave rise to right and left pulmonary arteries which thence coursed to enter their respective lungs an-

terior to the main bronchi. A widely patent ductus arteriosus measuring 1.3 cm. in circumference was situated in its usual position.

The left heart was markedly hypoplastic. Two pulmonary veins entered a very small left atrium, measuring approximately 1.2 cm. in its greatest diameter. The left atrio-ventricular orifice was absent, being obliterated by a thin grayish-white fibrous tissue septum measuring approximately 0.1 cm. in thickness. No chordae tendineae or remnants thereof were attached to this septum. There was a patent foramen ovale communicating with the right atrium, affording the only means of escape of blood from the left atrium. The left ventricle was an extremely small slit-like space measuring 2.5 cm. in length and a few millimeters in diameter. It appeared to lie in the left lateral wall of the right ventricle. It communicated with the latter by a defect in the membranous portion of the interventricular septum. It also communicated with the ostium of the aorta, which occupied a "rider" position with respect to the interventricular septum. Somewhat more than ¾ of the aortic ostium was situated over the right ventricle. It was guarded by 3 well-developed semilunar cusps, adjacent to 2 of which were the coronary ostia. It should be emphasized that the aortic orifice and the ascending aorta were of normal caliber. The aortic arch followed a normal course, giving rise to the arch vessels in the usual manner. Beginning immediately distal to the left subclavian artery, the aortic isthmus was markedly narrowed to measure 0.4 cm. in circumference. The narrowing of the isthmus abruptly terminated immediately proximal to the widely patent ductus arteriosus, and beyond this point, the aorta was normal in size.

Summary of Anatomic Features (See Fig. 3). 1, Complete atresia of the mitral valve. 2, Markedly hypertrophic and dilated right cardiac chambers. 3, Markedly hypoplastic left cardiac chambers. 4, Patent foramen ovale. 5, The pulmonary artery arose from the right ventricle, as did the aorta for the most part, the latter occupying a "rider" position. 6, Normal systemic and pulmonary venous returns to the heart. 7, Widely patent ductus arteriosus. 8, Marked aortic coarctation of the infantile type.

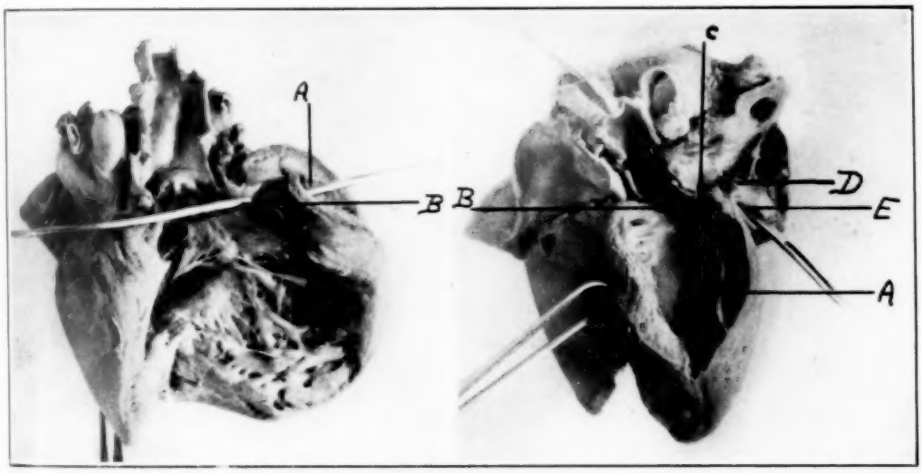

FIG. 1. Case No. 1. Heart showing aorta arising from greatly enlarged right ventricle. Probe is lying in the diminutive slit-like left ventricle (A), and extends through the interventricular septal defect (B), the upper margin of which is severed by the dissection.

FIG. 2. Case No. 1. View showing diminutive left ventricle (A), the lower margin of the interventricular septal defect (B), and the thin connective tissue septum ("atretic mitral valve") (C), separating the small left auricle (D) from the left ventricle. The probe is inserted into the patent foramen ovale (E).

Comment. It follows, then, that the systemic venous blood entered the right atrium, where a mixture with oxygenated blood from the left atrium occurred. The blood from the latter arrived through the patent foramen

ovale. From the right atrium, the mixed oxygenated and unoxygenated blood flowed to the hypertrophied right ventricle, and thence into both the pulmonary artery and aorta. Because both vessels had a common ventricular origin, the systolic pressures in each were equal. A portion of the blood in the pulmonary artery passed into the lungs, and the remainder flowed through the ductus arteriosus into the descending aorta. Since the ductus arteriosus was much larger than the aortic isthmus, by far the greater volume of blood delivered to the descending aorta arrived through the ductus. The blood passing from the single functioning ventricle into the ascending aorta supplied mainly the coronary arteries, and the vessels of the neck, head, and upper extremities. It would seem that the extremely small size of the left atrium would indicate that it served no greater function than that of a vessel through which oxygenated blood passed to the right atrium by way of the patent foramen ovale. Therefore we are dealing with an anatomically quadriloccular heart which is functionally biloccular.

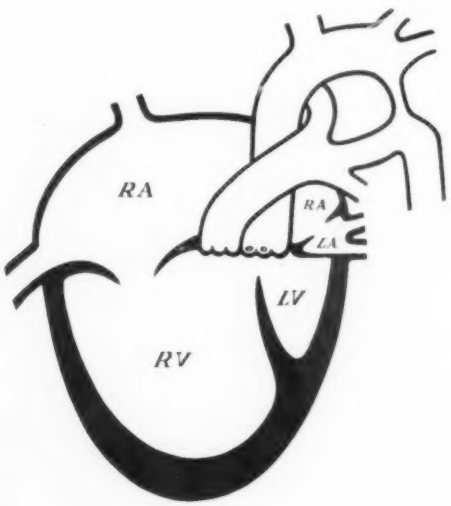

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FIG. 3. Case No. 1. Schematic diagram of heart and great vessels showing also the patent ductus arteriosus and coarctation of the aorta. RA—right auricle; LA—left auricle; RV—right ventricle; LV—left ventricle.

CASE NO. 2. *Clinical Summary.* A three-day old white male infant was delivered by forceps following a normal full term gestation. Immediately after birth it appeared to be normal. Its course was uneventful during the subsequent 36 hours, after which it suddenly developed a moderate cyanosis. At this time, a "machinery-like" murmur was heard in the pulmonic area. The cyanosis persisted despite continuous oxygen therapy for 40 hours, after which the infant expired.

Autopsy Findings. The body was that of a well-developed and well-nourished white male infant. The pertinent findings were limited to the heart and the great vessels.

The heart (See Figs. 4, 5 and 6) weighed 39 gm., the normal at the age of 3 days being 18 gm.[2]. It occupied a central thoracic position, and was somewhat globular in shape with a moderately rounded apex. The venae cavae terminated normally in an extremely dilated right atrium, estimated to be about 5 times the normal size. There was

a moderate hypertrophy of the trabeculae of the inner atrial wall. The orifice of the coronary sinus was not remarkable. The right atrio-ventricular valve measured 5 cm. in circumference, and its leaflets, which appeared normal, were attached to markedly hypertrophied ventricular papillary muscles by normal chordae tendineae. An extremely dilated right ventricle, the myocardium of which measured 0.5 cm. in thickness, was the only functioning ventricle. The pulmonary ostium, occupying a normal position, was

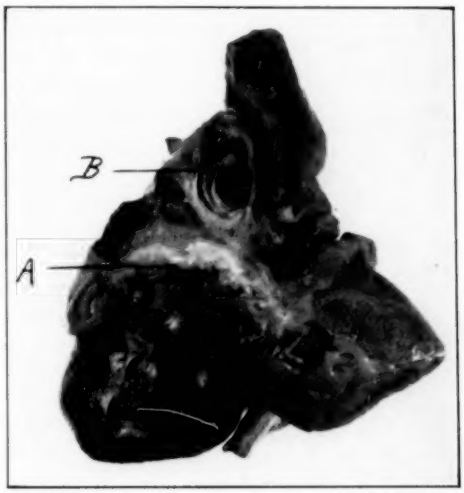

FIG. 4. Case No. 2. Heart showing hypertrophied and dilated right chambers, tricuspid valve (A), and patent foramen ovale (B).

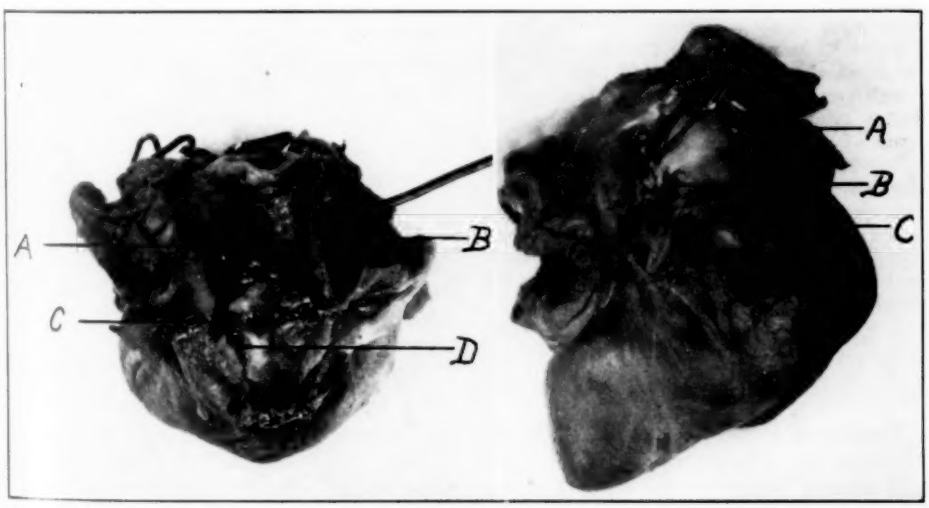

FIG. 5. Case No. 2. View showing hypoplastic left atrium (A), and probe extending through patent foramen ovale from right atrium (B), stenotic mitral valve (C), diminutive left ventricle (D).

FIG. 6. Case No. 2. View showing hypoplastic aorta (A) with stenotic fusion of the cusps (B). Proximal portion of right coronary artery is laid open (C).

guarded by 3 well-developed semilunar cusps. The ostium, as well as the first portion of the pulmonary artery, measured 2.5 cm. in circumference. The pulmonary artery divided into right and left pulmonary arteries which entered their respective lungs anterior to the main bronchi. A widely patent ductus arteriosus, measuring 1.5 cm. in circumference, was in its usual position, and communicated with the distal portion of the aortic arch.

The entire left heart was hypoplastic. Four pulmonary veins, 2 from each lung, entered a very small left atrium measuring 2 cm. in circumference. A widely patent foramen ovale measuring 0.7 cm. in diameter communicated with the right atrium. A small left atrioventricular orifice, measuring 1.5 cm. in circumference, possessed poorly developed and short leaflets attached by a few short chordae tendineae to the wall of a slit-like space, which represented an extremely small left ventricle. This space, measuring not over 1.5 cm. in its greatest dimension, appeared to lie in the left lateral wall of the large right ventricle. No outlet from the left ventricle could be identified.

The aorta was opened in either direction from the entrance of the ductus arteriosus. Proximally, it gave rise to each of the arch vessels in their normal positions. The ascending aorta measured only 1 cm. in circumference, and ended blindly at the base of the heart behind the pulmonary artery. The fused commissures and leaflets of the atretic aortic valve were easily seen. From the sinuses of Valsalva so formed, the orifices of the coronary arteries were identified, and each of these vessels pursued normal courses. Distal to the ductus arteriosus the descending aorta measured 1.6 cm. in circumference.

Summary of the Anatomic Features. 1. Markedly hypertrophic and dilated right cardiac chambers. 2, Markedly hypoplastic left cardiac chambers with a stenotic (not atretic) mitral valve. 3, Patent foramen ovale. 4, Only one efferent vessel, the pulmonary artery, which arose from the right ventricle. 5, Normal systemic and pulmonary venous returns. 6, A widely patent ductus arteriosus. 7, Atresia of the aortic ostium and a hypoplasia of the ascending aorta.

Comment. In this instance, as in Case 1, there was admixture of oxygenated and venous blood in the right atrium. The blood then flowed to the right ventricle, and thence into the pulmonary artery, where one portion passed to the lungs, while the remainder flowed through the ductus arteriosus to the aorta. Since the aortic ostium was completely closed, it is evident that a portion of the blood derived from the ductus arteriosus flowed in retrograde direction into the arch and ascending aorta to supply the arch vessels and coronary arteries, while the remainder flowed in normal direction distal to the insertion of the ductus. The pulmonic and systemic venous returns to the heart were normal. As in Case 1, it would seem that the extremely small size of the left atrium would indicate that it served no function other than that of a vessel through which oxygenated blood passed to the right atrium by way of the patent foramen ovale. With no outlet, the left ventricle could serve no function. Therefore, this case also presents an anatomically quadriloccular heart which is functionally biloccular.

Discussion. The fundamental defect in mitral atresia is a matter of some conjecture. Moenckeberg[12] was of the opinion that an atrioventricular septal shift-to-the-left occurred in embryo. He called these cases "cor pseudo-triloculare with rudimentary left ventricle." Though mentioned[3,7], we have found no convincing evidence that fetal endocarditis has ever been responsible for congenital mitral atresia. With one exception[18], the more recent writers do not refer to Moenckeberg's hypothesis, but rather do they believe that the fundamental defect is an overgrowth and fusion of the fetal endocardial cushions with consequent atresia of the mitral valve.

Harris and Farber[7] explained the aortic dextroposition and hypoplasia on the basis of Spitzer's phylogenetic theory. They supported their contentions statistically by showing that over half of the cases of mitral atresia, in itself a very rare anomaly, are associated with dextroposition of the aorta. The latter, also an unusual anomaly, is one in which mitral atresia is seldom seen.

They further stated that according to Spitzer's theory, the details of the frequently associated aortic malformations depend on the time in cardiac development at which mitral atresia occurred. The remaining defects, with one important exception, can be explained as a result of abnormal hemodynamics.

tutes a strong point in favor of Moenckeberg's atrio-ventricular septal "shift-to-the-left" hypothesis. Also, in our opinion, it defies adequate explanation on the basis of hemodynamics, for the intra-atrial pressure on the left must be greater than that on the right as is indicated by the direction of the blood flow from the left to the right atrium.

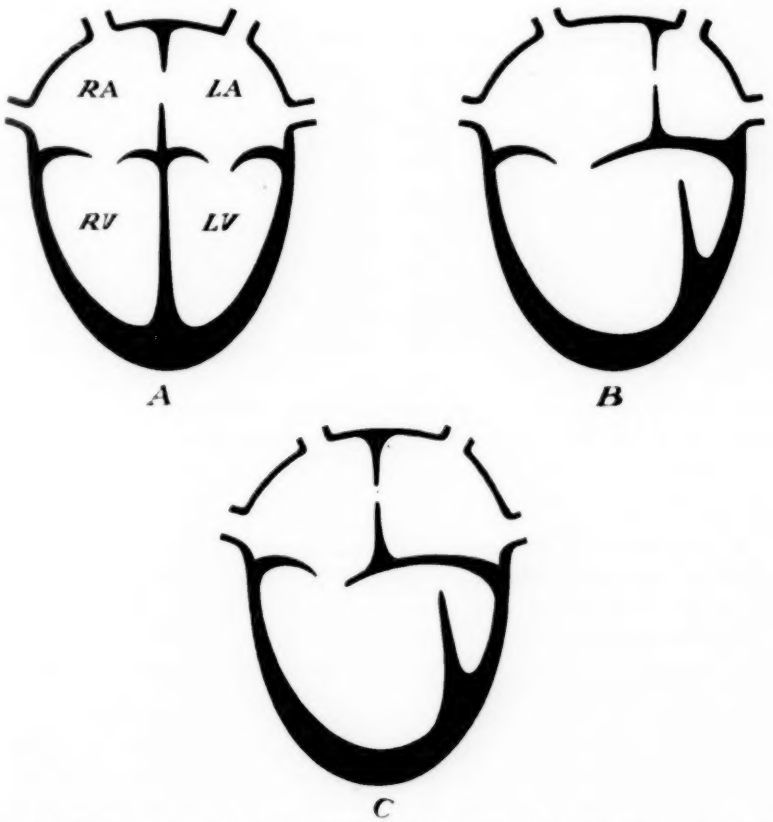

FIG. 7. Schematic diagrams of heart showing (A) normal, (B) usual configuration of chambers in mitral atresia (small left auricle); (C) configuration to be expected if the fundamental defect were a primary atresia of the mitral valve (normal or large left auricle).

One feature, however, the hypoplasia of the left atrium, is dismissed by current writers with a statement as to its existence. An analysis of case reports has brought to light that it occurred 20 times in 23 cases of mitral atresia[1,3,5,7,9-19], including our own. This Therefore, it would seem to us, one could justifiably maintain that if there were no inter-atrial septal shift-to-the left, the size of the left atrium should be at least normal, if not hypertrophied (See Fig. 7). The more simple Moenckeberg theory, however, explains

the small left atrium, the diminutive left ventricle, the hypertrophied right cardiac chambers, the mitral atresia, and the aortic dextroposition with the resulting inter-ventricular septal defect. Moreover, the identical configuration of the cardiac chambers in our second patient with a patent mitral valve would seem to support Moenckeberg's hypothesis by indicating that atresia of the mitral valve itself is not a necessary component of the anomaly, though it usually occurs. Similar cases have been encountered[3,5], although one of these[5] superficially resembled tricuspid stenosis due to bulbo-ventricular inversion[7].

The widely patent ductus arteriosus and the coarctation of the aorta in our first case are at least in part due to the aortic dextroposition, regardless of the underlying fundamental defect. It has been stated[8] that in fetal life the aortic isthmus has virtually no function, for the ductus arteriosus supplies considerable, if not all, of the blood to the aorta below the isthmus of the latter. The blood entering the ascending aorta from the heart is thus destined to supply the coronary arteries, the upper extremities, head and neck. Normally, at birth the aortic isthmus is somewhat smaller than the segments of aorta immediately proximal or distal to it. The subsequent increase in diameter of the isthmus to the size of the immediately adjacent aortic segments is apparently dependent upon the necessity imposed upon it by the closure of the ductus arteriosus after birth, for then the blood reaching the lower aorta must arrive through the isthmus. The closure of the ductus arteriosus is believed to result at least in part from a fall in blood pressure in the pulmonary artery incident to expansion of the lungs at the time of birth[4,8,17]. In the first case herewith reported, no such difference in blood pressure between the pulmonary artery and aorta could occur, for both vessels arose from the single functioning ventricle. It would then be logical to assume that the ductus arteriosus would remain patent. Furthermore, as a more widely patent vessel than the aortic isthmus, it would be expected to continue to supply the more substantial portion of the blood going to the descending aorta. This contention is also supported by the fact that in nearly all reports of congenital mitral atresia there has been an associated widely patent ductus arteriosus. Two-thirds, or 23 case reports, including our own, were available to us in the literature[1,3,5-7,9-19]. Of this number, it was stated that the ductus arteriosus was patent in 17. The absence of the ductus was definitely described in only one instance[1], in which there was an associated hypoplasia of the pulmonary artery. Walls[19] misinterpreted the vascular configuration in his case as a persistent truncus arteriosus. It is clearly evident from his description that he was dealing with an aortic valve atresia, and a hypoplasia of the first portion of the aorta which then must have supplied the coronary arteries by retrograde flow from a widely patent ductus arteriosus, a situation analogous to our Case 2. Although the ductus is not specifically referred to in Moenckeberg's description of Fraenkel's case, there is no question as to its existence as a patent vessel supplying blood to a hypoplastic aorta with an atretic aortic ostium. In Harris and Farber's Case 7b[7], one can only assume that the ductus was patent, since they stated that the findings were identical with those in their Case 7a. The information recorded in the case of Gibson and Clifton [6], and Case 30, of Taussig [17], is not sufficient to permit any conclusion concerning the status of the ductus. Therefore, of these 23 cases the existence of a patent ductus is known in 19 instances, doubtful in 1, absent in 1, and unknown in 2. This incidence

of a patent ductus in mitral atresia is in marked contrast to the fact that mitral atresia rarely occurs in the frequently encountered instances of patent ductus arteriosus.

In our second case, as well as others with an atretic aortic ostium, it is readily apparent that the ductus would remain open.

Mitral atresia is not a disorder which permits accurate antemortem diagnosis. It is classified by White[20] as a cyanotic type of malformation. The clinical signs and symptoms are not sufficiently characteristic to differentiate the disorder from other forms of *Maladie Bleue*, and moreover, are further modified by the signs and symptoms of other variable associated anomalies. However, the possible existence of such unusual, though hopeless, cases as those of mitral atresia must be considered when an infant is to be categorized as a surgically treatable or non-treatable patient.

Summary. 1. Two cases of congenital atrio-ventricular septal shift-to-the-left, one with mitral atresia, are reported.

2. From the relationships between mitral atresia, or stenosis, and the associated anomalies it is concluded that hypoplasia of the left atrium lends strong support to Moenckeberg's shift-to-the-left hypothesis.

3. A patent ductus arteriosus is so commonly associated with congenital mitral atresia that it may be taken to be an intrinsic part of the composite anomaly.

4. Previously offered explanations for the pathogenesis of the patent ductus arteriosus and the infantile type of aortic coarctation are consistent with the findings in our cases.

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IRRADIATION OF THE PITUITARY GLAND IN HYPERTENSIVE VASCULAR DISEASE*

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Favorable reports[6,7] of recent investigators have stimulated renewed interest in the use of irradiation of the region of the pituitary gland for treatment of essential hypertension. They applied a modification of the Burn method[1] of biological assay for the pituitary antidiuretic hormone as an aid to selection of candidates for treatment. The pituitary area was irradiated through 2 portals for 10 treatments with a total dosage of 2000 r. measured in air. They reported about 50% of their patients were benefited either with a significant fall in blood pressure or a relief of symptoms. It is because of these reports than we have undertaken this study.

Method. Selection of Patients. Forty-three hypertensive patients, ranging in age from 27 to 65 years were used in this study. Twenty-five were treated and 18 were used as controls. All patients received extensive studies in an attempt to evaluate their hypertensive status and also to eliminate those with hypertension of known etiology. A detailed history and physical examination was done by at least 2 examiners. Blood studies consisted of complete blood count, Kahn, and non-protein nitrogen tests. Kidney status was ascertained by urinalysis, urea clearance test, urine concentration tests, and intravenous pyelography. Cardiac status was further evaluated by electrocardiograms and roentgenological examination. The size of the sella turcica was estimated by roentgenograms of the skull. In addition, selected patients had cold pressor, sodium amytal, tetra-ethyl-ammonium chloride and benzo-dioxane studies.

All patients received a bioassay for the antidiuretic hormone by a modification of the Burn method[1] as an index of activity of the posterior pituitary gland. All treated patients received irradiation to the region of the pituitary gland, regardless of the bioassay results. Eleven of these patients had gonadotropic hormone assays prior to treatment. Only 2 of these follicle-stimulating hormone assays were abnormally elevated.

Bioassay Techniques. Our modification of the Burn technique is as follows: White laboratory rats of known strain weighing from 250 to 400 gm. were used. No attempt was made to restrict food or fluids preceding the tests. The rats were given 5 cc. of tap water per 100 gm. of body weight at room temperature via stomach tube. One cubic centimeter of patient's serum was injected intra-peritoneally through a No. 23 needle. The rats were given a whiff of ether at the beginning and the end of the assay to insure complete emptying of the bladder. The rats were placed for a period of 90 minutes in metabolism cages and the urine collected in calibrated tubes.

A positive test was one in which the rat retained 4 cc. or more of water per 100 gm. body weight; if the retention was less than 4 cc. per 100 gm. body weight, the test was recorded as negative.

Three rats were used in each assay. The

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TABLE 1. RESULTS OF PITUITARY IRRADIATION IN 25 HYPERTENSIVE PATIENTS

			Before Treatment			Follow Up Period In	After Treatment			Significant Lowering of	Significant Symptomatic
Pt.	Sex	Age	Assay	Blood Pressure*	Symptoms**	Weeks	Assay	Blood Pressure*	Symptoms**	Blood Pressure	Improvement
E.S.	F	44	+	192/127	++	90	—	187/116	++	No	No
F.D.	F	56	+	222/108	+++	30	—	221/107	+	No	Yes
V.G.	M	46	+	162/107	+	34	—	175/117	0	No	Yes
J.S.	F	40	+	223/114	++	24	—	229/136	+	No	Yes
O.F.	F	55	+	206/117	++	66	—	182/112	0	No	Yes
A.P.	M	41	+	214/127	++	20	—	215/121	0	No	Yes
R.M.	M	58	+	213/108	++	20	—	197/95	0	No	Yes
M.B.	F	39	+	188/127	+++	18	—	204/128	+++	No	No
M.C.	F	46	+	206/122	+++	6	—	170/120	+++	Yes	No
T.M.	F	49	+	226/131	+++	16	—	218/129	+	No	Yes
C.O.	F	44	+	204/103	++	17	—	209/106	0	No	Yes
L.M.	F	48	+	188/101	++	15	—	174/92	+	No	Yes
C.B.	M	63	+	170/106	++	12	—	216/118	0	No	Yes
E.M.	F	49	+	243/124	+++	82	+	245/114	+	No	Yes
L.H.	M	46	+	186/108	++	60	+	190/107	+	No	Yes
A.S.	F	53	+	159/97	++	16	+	164/97	++	No	No
L.M.	M	46	+	169/119	+	12	+	153/97	0	Yes	Yes
L.P.	M	59	—	183/99	+++	84	—	183/98	+	No	Yes
S.M.	F	38	—	165/107	++	82	—	170/109	++	No	No
E.B.	F	47	—	197/114	++	20	—	202/114	+	No	Yes
L.C.	M	57	—	189/126	++	12	—	191/123	++	No	No
C.W.	F	41	—	200/130	+++	20	—	195/122	+	No	Yes
S.B.	F	57	—	177/112	++	16	+	147/93	0	Yes	Yes
J.G.	M	29	+	225/159	+++	8		240/167	+++	No	No
W.G.	F	33	—	189/134	+++	23		224/147	+++	No	No

* Represents the mean of 10 different determinations.

** 0 representing no symptoms to 3+ severe symptoms.

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assay was recorded as positive when at least two of these rats had a positive test.

Prior to use in actual testing of patients serum each rat fulfilled 2 requirements: 1, the animal had a negative test when given the usual amount of water without any intraperitoneal injection; 2, the animal had a positive test when given the usual amount of water by stomach tube and 5 milli units of pitressin* per 100 gm. of body weight intraperitoneally. These restrictions resulted in the rejection of 15 rats out of a colony of 60.

Pituitary gonadotropic activity was determined by the usual laboratory bioassay methods[2,5].

ROENTGEN-RADIATION FACTORS. A 200 kilovoltage constant potential therapy machine at 15 milli amperes was used. Target-skin distance was 50 cm. The half value layer with 2 mm. of copper added filtration was 2.4 mm. of copper. The area irradiated equaled 48 sq. cm. and consisted of 2 lateral temporal fields. Twenty-five roentgens were delivered per minute in air. One field a day was treated with 200 roentgens per treatment. The total number of roentgens delivered in air to each field was 1000. The treatments were given at 24-hour intervals. The pituitary area received a calculated dose of 1100 roentgens in 10 days.

Control patients were first prepared by marking off the lateral temporal fields as used in actual treatments. These areas were then leaded off and the port placed in position. The filament circuit was energized and the shutter was opened, simulating as nearly as possible an actual exposure to Roentgen-rays. However, no irradiation was given, though the patients had no reason to suspect they were not actually receiving therapy.

MEASUREMENT OF BLOOD PRESSURE. All blood pressure readings during the period of observation were taken by one of 2 examiners in the same examining room. Patients were given a 15-minute rest period, then the blood pressure was recorded from both arms while sitting in an upright position, using the same blood pressure instrument at each visit. At no time were patients told of the actual level of the blood pressure or any indication given as to the trend. Patients were seen daily during the 10 days' therapy, followed by weekly visits for approximately 3 months, when visits were lengthened to monthly intervals. Also at each visit the patient was questioned as to symptoms of vertigo, headache, dyspnea, and general sense of well being. The effect of treatment upon these 4 symptoms was the criterion for judging symptomatic response.

* Parke, Davis and Company.

Results. BLOOD PRESSURE RESPONSE. The effect of treatment upon mean blood pressure will be noted in Table 1. A lowering of 30 points in the systolic pressure or 20 points in the diastolic has been considered significant by Hutton[3] in studies of adrenal and pituitary irradiation for hypertension. If this arbitrary criterion is applied to this series, 3 (12%) of the treated and 3 (16.6%) of the control patients had a significant reduction in blood pressure.

EFFECT ON SYMPTOMS. Seventeen (68%) of the treated patients and 14 (77.7%) of the control group experienced symptomatic improvement.

RETINAL CHANGES. The Keith, Wagner and Barker[4] classification of hypertensive retinopathy was used in this study. Funduscopic examination was done on 24 patients. One patient had bilateral lens opacities which prevented examination of the retina. The results appear in Table 2.

TABLE 2. RETINAL CHANGES FOLLOWING IRRADIATION OF PITUITARY IN HYPERTENSION

Number of Patients Before Treatment	Classification	Number of Patients After Treatment
3	0	0
7	I	7
11	II	13
2	III	2
1	IV	2

ELECTROCARDIOGRAPHIC CHANGES. Serial electrocardiograms recorded on the treated patients, before, during, and at completion of therapy, revealed a definite trend from their normal toward a left strain pattern in 4 patients (L.C., C.O., W.G., and L.M.). Thus, 16% showed progressive changes. In no patient did the electrocardiogram revert toward a more "normal" pattern.

BLOOD NON PROTEIN NITROGEN. Repeated blood non-protein nitrogen determinations showed a definite change from normal to abnormal in 5 patients (E.M., E.S., M.C., L.M., and J.S.). Patient J.S. was the only one in whom the pre-treatment blood non protein nitrogen was abnormal. It increased from 59 to 75 mg. per 100 cc. during treatment. Thus, 6 patients (24%) had sustained elevation of the blood non-protein nitrogen following treatment.

CHANGES IN HEART SIZE. There was no reduction in heart size of any of the treated patients as determined by the cardio-thoracic ratio on a 2 meter roentgenogram of the chest.

Discussion. In only 3 of the treated patients was there a suggestion of significant lowering of the blood pressure. In order critically to judge the treatment, 2 tests for statistical significance were made on the mean average blood pressures before and after irradiation. Both the Standard Error and the Critical Ratio tests applied to the changes in systolic and diastolic pressures showed that the differences which occurred could be purely chance and were not statistically significant when considered as a group.

Symptomatic improvement occurred in the majority of both treated (68%) and control (77.7%) patients. There is no correlation between changes in the antidiuretic assay following irradiation and symptomatic improvement of the treated group. We have no explanation for the symptomatic improvement other than the psychological effects of modern Roentgen therapy.

Initially, 18 of our patients had a positive serum anti-diuretic assay. Following irradiation the assay changed to negative in 13 cases. In 4 it remained positive, and 1 patient died without re-assay. This would indicate that in the majority of cases it is possible to convert a positive anti-diuretic assay to a negative with Roentgen therapy in this dosage.

Four patients in this series died following irradiation. Two of these patients (J.G., and M.C.) had malignant hypertension and showed no response to treatment. One patient (W.G.) died of spontaneous subarachnoid hemorrhage. The only patient (E.S.) in whom autopsy permission was secured, died of an acute homologous serum jaundice secondary to plasma administration while on another service. Microscopic sections of the pituitary gland showed no changes which could be ascribed to the Roentgen therapy received 90 weeks before death. Patient E.M. received 2 complete courses of Roentgen therapy in a period of 5 months without effect on assay or blood pressure. The only complication noted as a result of therapy was a loss of hair in the temporal region of some of the treated patients.

Summary. 1. A group of 25 patients, followed for a period of 6 weeks to 3 years, with essential hypertension, was treated with 2000 r. units (measured in air) to the region of the pituitary gland, as previously recommended, without significant lowering of blood pressure when considered statistically as a group. Seventeen patients (68%) had symptomatic improvement. Three patients (12%) showed a fall in blood pressure of 30 mm. Hg. systolic or 20 mm. Hg. diastolic, but no other objective evidence of improvement was noted.

2. The 18 control patients were managed in an identical manner with the treated group with the exception that Roentgen therapy was not actually given. Fourteen of the 18 patients (77.7%) were improved symptomatically. Three of these patients (16.6%) showed a fall in blood pressure of at least 30 mm. Hg. systolic or 20 mm. Hg. diastolic.

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RENAL LESIONS ASSOCIATED WITH CHRONIC ULCERATIVE COLITIS*

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Although the essential lesions of chronic ulcerative colitis reside in the colon, this disease may cause widespread and important pathologic changes in other parts of the body. This is illustrated by the occurrence of lesions involving the skin, the joints, the vascular system, the eye and other organs[1]. In addition, marked disturbances in the metabolism of minerals, vitamins and foodstuffs in general are revealed by laboratory studies[4]. These pathologic processes are well recognized and have been described in the literature[6,7,9] even though, in many or most instances, the mechanisms involved have not been fully understood. It is evident, however, that the systemic effects of this form of ulcerative colitis are many and varied.

The investigation to be reported herein was undertaken to determine what changes, if any, occur in the kidneys of patients with chronic ulcerative colitis, and to correlate, when possible, such changes with the clinical picture.

Jankelson, McClure and Sweetsir[6], in an excellent study of the complications occurring outside the gastro-intestinal tract in a series of 145 cases of chronic ulcerative colitis, reported involvement of the urinary tract as follows: 1, acute nephritis, 12 cases; 2, nephrosis, 1 case (the basis for this diagnosis was not stated); 3, pyelitis, 3 cases; 4, cystitis, 3 cases; these cases of cystitis and pyelitis occurred in females; and *Escherichia coli,* staphylococci and streptococci alone or in combination were found to be the infecting organisms; and, 5, renal stones, 2 cases. The diagnosis of acute nephritis was made on the basis of fixed specific gravity, diminished output of urine, and the presence of albumin, casts and erythrocytes in the urine. As a rule, the blood pressure was not elevated and the retinas were normal. The presence or absence of edema in the 12 cases of acute nephritis was not mentioned, although the authors reported some degree of edema present in 76% of the 145 cases. This edema was attributed to malnutrition. Azotemia was present in some cases, although, as the authors pointed out, the relationship of the azotemia to the nephritis was obscured by the fact that azotemia may be present not only in toxic and dehydrated patients but also in patients with uncomplicated chronic ulcerative colitis. The authors stated that since the cause of acute nephritis is toxemia, it is apparent that nephritis occurrs in the acute stage of the primary disease and is likely to subside with it. They stated further that they had not observed the acute nephritis to pass into chronic

* Abridgment of thesis submitted by Dr. Jensen to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

nephritis. It was their belief that in some cases the nephritis contributed to the mortality.

Ricketts and Palmer[9], in a study of the complications of chronic ulcerative colitis, analyzed data on 206 cases. They reported 2 cases of pyelonephritis and 1 case of renal stones. Nephritis was not mentioned as a complication.

Lindahl and Bargen[8] reported on 6 cases of nephrolithiasis complicating chronic ulcerative colitis after ileostomy had been performed in a series of 182 cases. They concluded that renal calculi occur more frequently after ileostomy in those cases in which the colon is not removed than in those cases in which the colon is removed after the ileostomy. They stated their belief that the basis for the formation of the calculi might be urinary infection secondary to the remaining diseased colon. In 3 of their cases they found *Aerobacter aerogenes*, and in the other 3 cases they found *Escherichia coli*, *Aerobacter aerogenes* and *Staphylococcus aureus*, in combination, as the infecting agents. They pointed out also that the colon, if not removed after an ileostomy performed because of chronic ulcerative colitis, remains as a focus for the possible development or continuation of the complicating lesions such as arthritis, erythema nodosum, and so forth.

Schlicke and Bargen[10] reported that infection of the urinary tract was noted in only 1 of the 10 cases of chronic ulcerative colitis in which necropsy was performed.

Method of Investigation. To determine the presence of infection of the urinary tract, cultures of catheterized specimens of urine from 22 unselected hospital patients with chronic ulcerative colitis were made. To determine impaired renal functions, urea clearance tests were performed on 16 unselected hospital patients with chronic ulcerative colitis. In the very sick patients this test was delayed somewhat until the patient was over the acute phase of his illness. Unfortunately, it is in the acute phase that this test would be expected to show the greatest change from normal in kidney function.

The pathologic material included the kidneys in 60 consecutive cases of chronic ulcerative colitis in which postmortem examination was done and in which the diagnosis was definitely established clinically and pathologically.

Two groups of controls were used: 1, normal control kidneys obtained from 60 patients who died of brain tumors, poliomyelitis and accidental causes, and in whom there was no evidence of infection anywhere in the body, and, 2, kidneys from 35 patients who died of peritonitis secondary to miscellaneous gastro-intestinal disorders, but excluding patients with any type of enteritis or colitis. This group of controls with peritonitis was used because 31 of the 60 patients with chronic ulcerative colitis had peritonitis to a varied extent.

Results. Renal Function. Of the 16 unselected hospital patients with chronic ulcerative colitis on whom urea clearance studies were carried out, all (7) with grade 1 activity of the colitis (graded on the basis of 1 to 3) showed normal urea clearance values. Three patients with grade 2 and 3 with grade 3, activity of the colitis showed lowered urea clearance values, in the range considered to be of questionable significance. Values in this range may represent extreme, but normal, fluctuations.

Three of the 16 patients showed definitely impaired renal function, with marked lowering of the urea clearance values. In 1 of the patients with definitely impaired function, a urea clearance test performed 2 months later disclosed a return of the urea clearance value to normal. Of these 3 patients with definitely impaired renal function, 2 had grade 3 activity of the colitis, whereas the third had grade 2 activity of the colitis.

Weight of Kidneys. It has been suggested that ileostomy might, in some way, influence the weight of the kidneys. Table 1 compares the frequency of renal hypertrophy in cases of chronic ulcerative colitis with ileostomy with the frequency in cases without ileos-

TABLE 1.—RENAL HYPERTROPHY IN CHRONIC ULCERATIVE COLITIS

	Kidneys, pairs		
		Weight over 350 gm.	
Type of Case	*Total*	*Number*	*Per cent*
With ileostomy	20	9	45.0
Without ileostomy	40	9	22.5

tomy. The arbitrary value of 350 gm. or more for the combined weight of the kidneys was established to represent renal hypertrophy. It is acknowledged that this value may be justly disputed. For the purpose of this study, however, the value of 350 gm. serves well as a basis of comparison.

TABLE 2.—PROLIFERATION OF THE ENDOTHELIAL CELLS OF THE GLOMERULAR CAPILLARIES

	Per cent in grade				*Total, grades*
Series	*0*	*1*	*2*	*3*	*1 to 3*
60 normal controls	91.6	6.6	1.7	0	8.3
35 controls with peritonitis	60.0	34.2	5.8	0	40.0
Chronic ulcerative colitis, 60 cases	30.0	36.6	21.6	11.6	70.0

Grading of endothelial proliferation: 0, none; 1, mild but definite; 2, moderate, and 3, high degree.

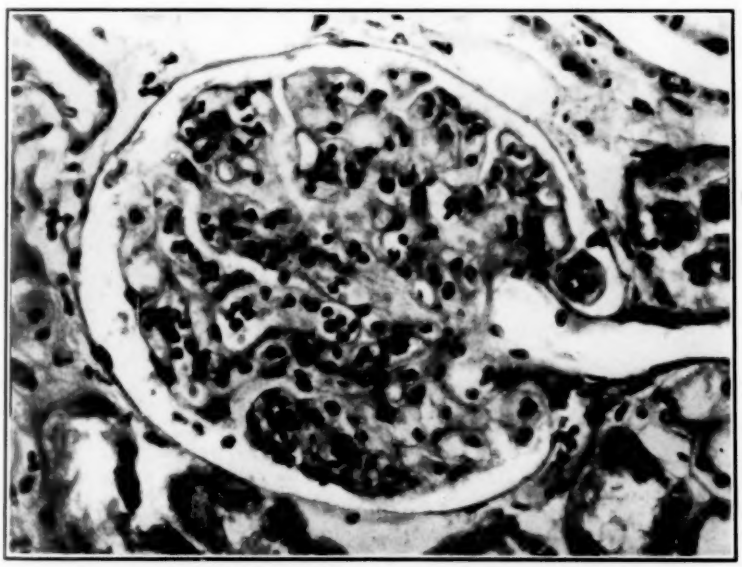

FIG. 1. Normal glomerulus (hematoxylin and eosin x320).

GLOMERULAR LESIONS. Proliferation of the endothelial cells of the glomerular capillaries was the significant lesion in the glomeruli (Table 2). A normal glomerulus is shown in Figure 1.

In grade 1 endothelial proliferation, there was no obstruction to the flow of blood through the glomerular capillaries (Fig. 2*a*). In grade 2 endothelial proliferation, there appeared to be moderate obstruction in some of the capillary loops and very little obstruction in other capillary loops (Fig. 2*b*). In grade 3 endothelial proliferation, there appeared to be marked obstruction to the flow of blood in the capillaries so that a majority of the capillaries appeared almost bloodless (Fig. 3). In

some of the glomeruli showing grade 3 endothelial proliferation, intracapillary fibrils could be seen. Occasionally, crescents also were observed in the cases in which there was grade 3 endothelial proliferation (Fig. 3*b*). Various degrees of swelling of the basement membrane were present in cases in which there was both grade 2 and 3 endothelial proliferation. In some cases, it seemed to reduce materially the lumen of the capillaries.

It is apparent from a study of Table 2 that in chronic ulcerative colitis a high percentage (70%) of the patients have endothelial proliferation of some

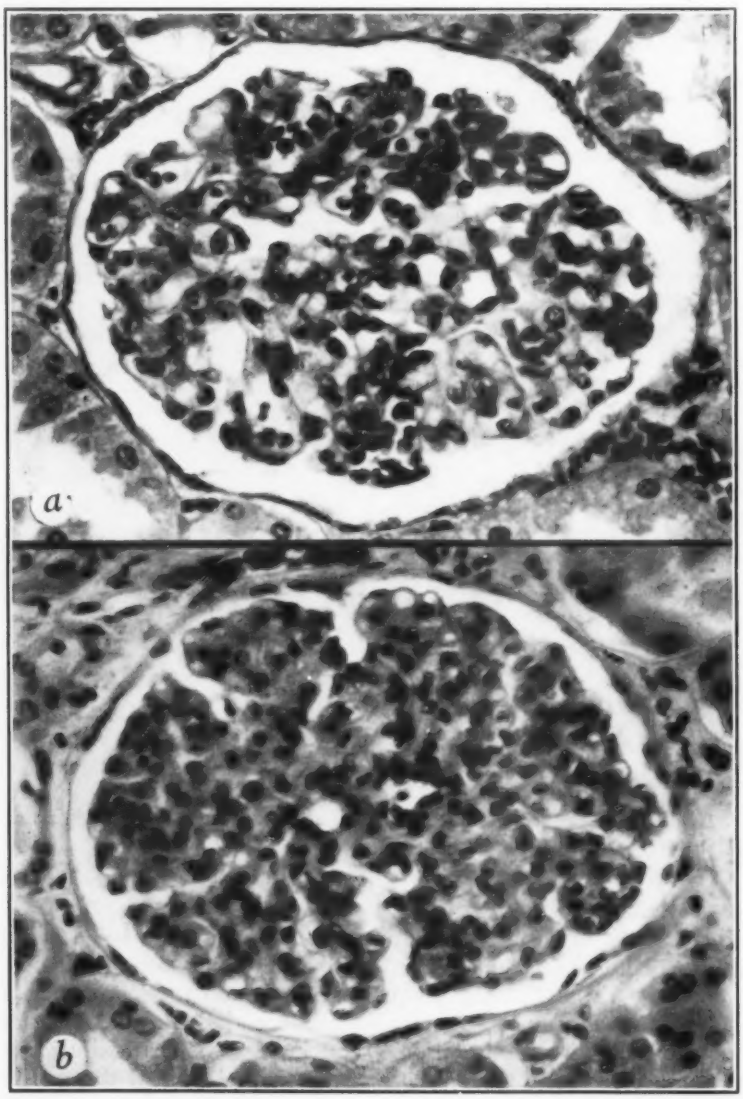

FIG. 2. Glomerulus with endothelial proliferation. *a*. Grade 1 (hematoxylin and eosin x435). *b*. Grade 2 (hematoxylin and eosin x400).

degree, and that in approximately 11% the endothelial proliferation (glomerulitis) is sufficient to cause marked obstruction to the flow of blood through the glomerular capillaries. This capillary obstruction approaches that seen in clinical acute glomerulonephritis. It may be noted that the percentage (11.6%) of patients having grade 3 endothelial proliferation is similar to the percentage (8.2%) of patients diagnosed as having acute nephritis in the series of 145 patients reported by Jankelson and co-workers[6].

Effect of Peritonitis. Peritonitis was present in about half of the cases of

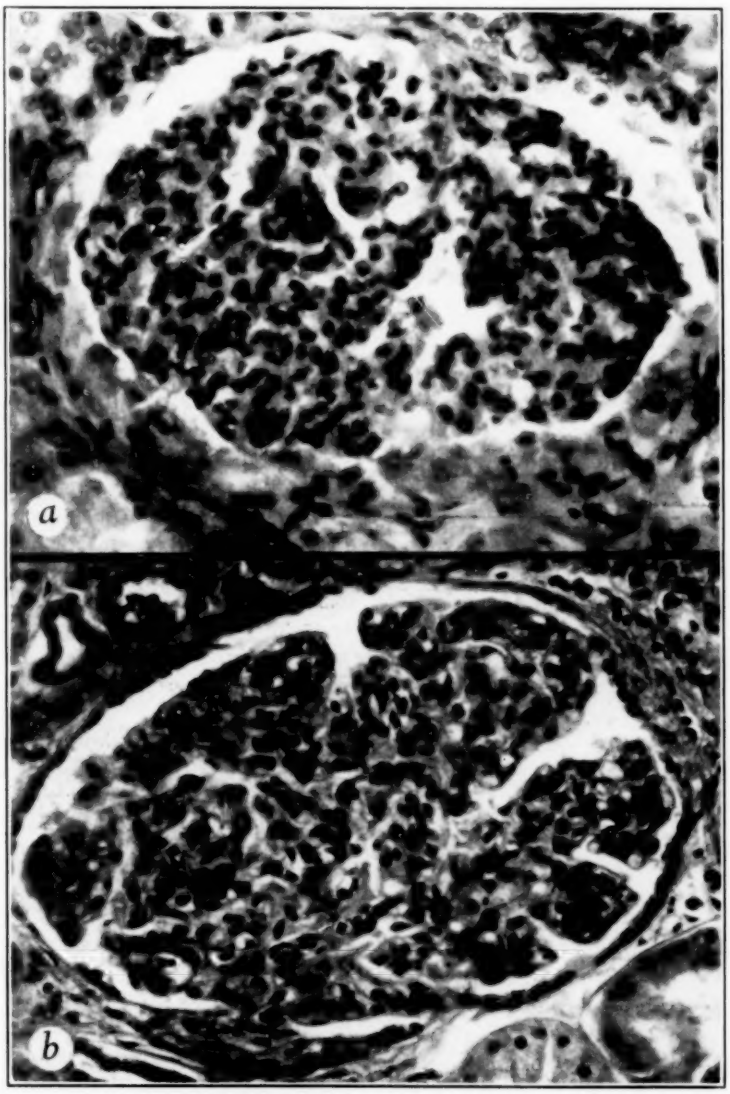

FIG. 3. Glomerulus with endothelial proliferation, grade 3. *a.* Hematoxylin and eosin x375. *b.* Note epithelial crescent (hematoxylin and eosin x375).

chronic ulcerative colitis. That it had little effect in the production of grade 2 and grade 3 endothelial proliferation but that it did have an effect in the production of grade 1 endothelial production was apparent. In the cases of chronic ulcerative colitis grades 2 and 3, endothelial proliferation occurred just as frequently in the patients without, as in the patients with, peritonitis. Grade 1 endothelial proliferation, however, occurred in twice as many patients with peritonitis as it did in patients without peritonitis. This observation is supported also in Table 2, where it is seen that in the *control* group of patients with peritonitis (without chronic colitis) almost all the patients with endothelial proliferation were in the grade 1 subgroup.

Effect of Type of Colitis. All patients with grade 3 endothelial proliferation were in the group of patients who had chronic ulcerative colitis (type 1 of Bargen). The patients with grades 1 and 2 endothelial proliferation were distributed equally between the chronic ulcerative and the segmental types of colitis.

Relation to Activity of Colitis. The degree of endothelial proliferation was roughly, but in direct manner, proportional to the activity of the colitis (Table 3).

TABLE 3.—RELATION OF ENDOTHELIAL PROLIFERATION TO THE ACTIVITY OF THE COLITIS

Activity of colitis, grade	*Total cases*	*Endothelial proliferation, grades 1–3*	
		Cases	*Per cent*
1 (mild)	11	5	45.4
2 (moderate)	37	25	67.6
3 (severe)	12	12	100.0

Cause of Endothelial Proliferation. Endothelial proliferation is a nonspecific reaction of the glomeruli to injury[5]. It occurs in a wide variety of infectious states and in certain noninfectious conditions[2,3]. Its occurrence in chronic ulcerative colitis may be considered as evidence of injury to which the glomerulus has responded in its usual manner.

Correlation of Clinical Data With Endothelial Proliferation. Because the presence of albumin and erythrocytes in the urine is indicative of glomerular damage, the clinical data in these necropsy cases are summarized here in connection with the findings on endothelial proliferation.

In 58 of the 60 cases of chronic ulcerative colitis (96.7%) albumin was present to some degree in at least 1 specimen of urine. The specimens in 11 cases gave values as high as grades 3 and 4 for albumin (on a basis of 1 to 4). In almost every case, there was considerable fluctuation from day to day in the amount of albumin. Some patients with grade 3 albuminuria on one day might have no albuminuria on other days. There was no correlation between the degree of endothelial proliferation and the amount of albumin in the urine.

In 19 of the 60 cases, erythrocytes were present in significant numbers at some time during the illness. In 16 other cases, they were present in minimal numbers.

Blood urea levels showed no general correlation with endothelial proliferation. This lack of correlation is probably related to the frequent disturbance of water and mineral balance in chronic ulcerative colitis and to the effect of such disturbances on renal function and levels of blood urea.

The presence or absence of edema has not been recorded because it would be impossible in most cases to separate the edema of renal origin from the edema due to the hypoproteinemia so frequently found in chronic ulcerative colitis.

In only 4 of the 60 cases was there hypertension. In each of these 4 cases, other conditions were present that could account for the elevated blood pressure. These conditions were 1, pregnancy, 2, amyloid contracted kidney, 3, chronic glomerulonephritis, and 4, senile arteriosclerosis. The depressing effect on the blood pressure of chronic debilitating diseases is well known and was borne out by this study.

Chronic glomerulonephritis was present in only 1 case. In that case, the occurrence, 1 year before death, of a sore throat followed by edema, anuria and a high degree of albuminuria suggests the throat infection, not the chronic colitis, as the important etiologic factor.

One patient in this series had acute bacterial endocarditis in addition to chronic ulcerative colitis of 3 years' duration. *Staphylococcus aureus* was isolated from the blood. Necropsy disclosed large vegetations on the leaflets of the aortic and mitral valves. The kidneys weighed 320 gm. and on microscopic examination showed endothelial proliferation, grade 2, as well as thrombi in the glomerular capillaries. No focal glomerular infarcts characteristic of subacute bacterial endocarditis were seen. There were, however, several early suppurative lesions in the cortex as well as several areas of tubular degeneration and necrosis.

TUBULAR LESIONS. 1. *Degeneration and Necrosis.* This lesion occurred in 14 cases of chronic ulcerative colitis but only to a minimal degree in 1 of the normal controls. Its incidence in the series of control patients with peritonitis and in those with chronic ulcerative colitis is summarized in Table 4. In most cases, this lesion was present to only a small or moderate extent. Table 4 reveals that if one considers only the number of cases of chronic ulcerative colitis with peritonitis, the incidence was approximately the same as in the control cases of peritonitis. Actually, however, in a number of cases of chronic ulcerative colitis in which there was no peritonitis this lesion was present. In several cases there were active regeneration and proliferation of cells lining the tubules, probably indicative of previous necrosis and degeneration.

TABLE 4.—INCIDENCE OF DEGENERATION AND NECROSIS OF THE TUBULAR EPITHELIUM OF THE KIDNEY

	Cases		
Type of case	*Total*	*Peritonitis*	*Degeneration and necrosis*
Chronic ulcerative colitis	60	31	14
Controls with peritonitis	35	35	11

2. *Vacuolization.* This lesion is characterized by the presence of large round clear areas in the cytoplasm of the tubular epithelium (Fig. 4). It occurred in 5 of the patients with chronic ulcerative colitis. The largest vacuoles filled the entire cell and pushed the nucleus to one side. In those cases in which the vacuoles were present to a lesser extent, they occurred almost entirely in the cells of the proximal convoluted tubules. Where the involvement was more extensive, it seemed that all portions of the tubules were involved, and in addition small areas of necrosis and degeneration were present. Stains for fat and glycogen gave negative results.

Vacuoles were not seen in any of the normal controls, and in only 1 of the controls with peritonitis were they present to a minimal degree.

The nature of these vacuoles was not determined. That they probably seriously interfered with renal function is indicated by the fact that in 4 of the 5 cases, terminal oliguria and anuria developed, lasting in the different cases from 3 to 6 days before death. Dehy-

dration was not a factor in the impaired urinary output, as all the patients had been receiving adequate amounts of fluid intravenously. A careful search of their records revealed no unusual medicine or form of treatment common to all these patients that might account for the lesion.

3. *Calcification of Tubules.* Scattered small deposits of calcium were present in 1 normal control, in 2 controls with peritonitis and in 9 patients with chronic ulcerative colitis. Most of the calcium deposits were in the smaller tubules, involving the loop of Henle. The significance of this lesion has not been

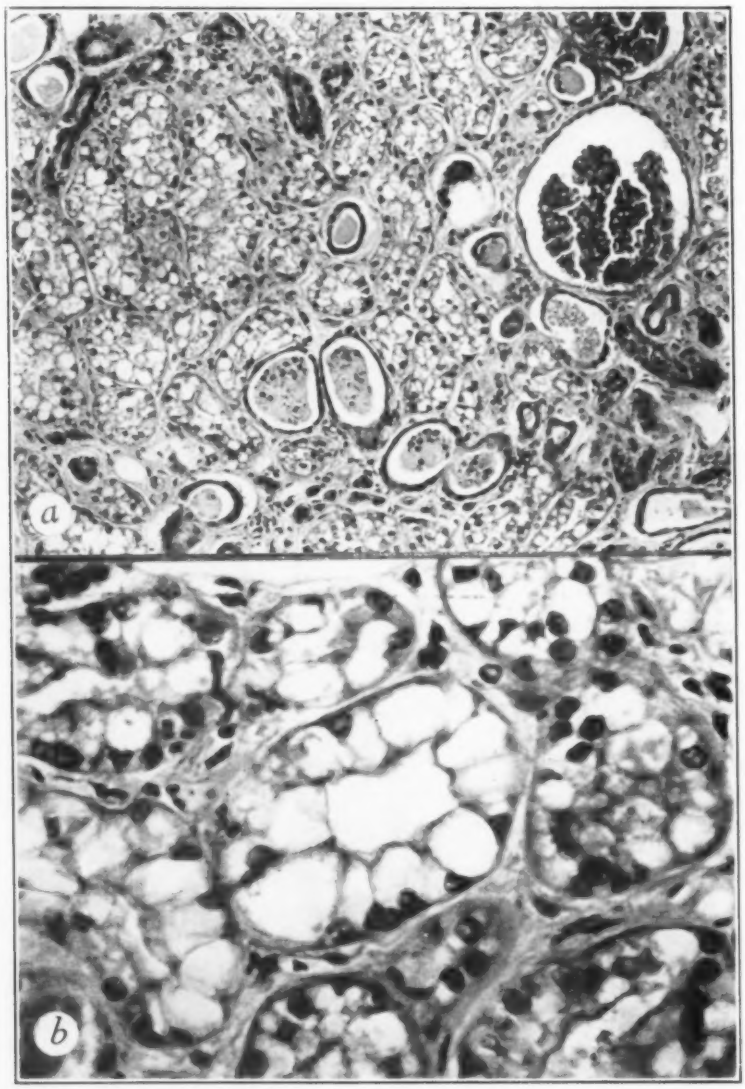

FIG. 4. Large vacuoles in epithelial cells of tubules. *a.* Hematoxylin and eosin x140. *b.* Hematoxylin and eosin x500.

established, but may represent previous degeneration and necrosis with subsequent healing and calcification.

4. *Pyelonephritis.* Acute pyelonephritis occurred in 4 cases of chronic ulcerative colitis and in 2 of the controls with peritonitis. In all cases, the infection probably occurred by way of the blood stream inasmuch as the pelvis of the kidney in each case showed little or no evidence of infection. In 1 other case there were bilateral ureteritis and pyelitis without infection involving the kidney, and this probably represented an ascending infection.

Neither cultures of the blood nor cultures of the urine were made in any of the 4 cases in which acute pyelonephritis occurred in this series.

Cultures of the urine made in 14 other cases of this series, however, gave positive results in 11 cases and negative results in 3. In none of the 11 cases in which the results were positive was there gross or histologic evidence of pyelonephritis.

Active chronic pyelonephritis was not seen in any cases of chronic ulcerative colitis in this series. There were 3 cases in which the lesions were suggestive of minimal involvement but the evidence was inconclusive.

A separate clinical study was carried out on 22 unselected hospital patients who had chronic ulcerative colitis with all degrees of severity of the disease. Catheterized specimens of urine were cultured on standard media. For 16 of the 22 patients the cultures gave negative results, whereas for 6 the results were positive as follows: Pseudomonas in 2 cases, micrococci in 2, *Escherichia coli* in 1, and *Streptococcus faecalis* in 1. None of these patients complained of urinary symptoms.

5. *Renal Calculi.* Nephrolithiasis occurred in only 1 patient with chronic ulcerative colitis. This patient had undergone ileostomy 1 year prior to death and a colectomy 2 days before death. Necropsy revealed bilateral pyelitis and ureteritis of a mild degree with a small calculus in the right renal pelvis. No obstruction was apparent.

This case is in addition to the 6 similar cases described in 1941 by Lindahl and Bargen[8] and referred to earlier in this paper.

Summary and Conclusions. Endothelial proliferation (glomerulitis) occurred in 70% of a group of 60 cases of chronic ulcerative colitis. The endothelial proliferation was a slight degree in 36.6%, of moderate degree in 21.6% and of high degree in 11.6% of the cases. Endothelial proliferation of high degree, with swelling, appeared to cause marked obstruction to the flow of blood in the glomerular capillaries. There was a direct correlation between the degree of endothelial proliferation and the degree of activity of the colitis. The highest degree of endothelial proliferation occurred in the cases of type 1 chronic ulcerative colitis (Bargen's classification). There was no correlation between the degree of endothelial proliferation and the presence of albumin or cellular elements in the urine. It was concluded that chronic ulcerative colitis is probably not an important factor in causing clinical chronic glomerulonephritis. Glomerular function as measured by urea clearance studies was definitely depressed in 3 and questionably depressed in 6 of the 16 patients studied clinically.

Tubular degeneration and necrosis of slight to moderate degree occurred in 23.3% of the 60 cases of chronic ulcerative colitis. Large vacuoles occurred in the tubular epithelium in 5 cases (8.3%). The cause and nature of these vacuoles were not determined. Four of the 5 patients with this lesion had terminal oliguria and anuria for a period of 3 to 6 days before death in spite of adequate therapy with fluids. Deposition of calcium in the tubular epithelium

occurred in 15% of the cases of chronic ulcerative colitis, as compared with its occurrence in 5.8% and 1.7%, respectively, in a group of controls with peritonitis and in a group of normal controls.

Acute pyelonephritis occurred in 4 (6.7%) of the 60 cases of chronic ulcerative colitis. Chronic pyelonephritis did not occur in any of these cases. The kidneys, in 11 cases in which cultures of urine showed bacteria some time before death, revealed no histologic evidence of pyelonephritis. Six of 22 unselected hospital patients with chronic ulcerative colitis had positive results on culture of the urine in the absence of urinary symptoms.

A renal calculus was present in 1 of the 60 cases of chronic ulcerative colitis. Its possible relationship to an ileac stoma made 1 year before death is discussed.

After ileostomy, there was a tendency to an increase in renal mass. In 45% of 20 cases in which ileostomy had been performed, the combined weight of both kidneys was more than 350 gm. It was concluded that ileostomy in chronic ulcerative colitis has no effect on the incidence or degree of endothelial proliferation or tubular lesions.

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THE EFFECT OF ORALLY AND INTRA-ARTERIALLY ADMINISTERED LYSOZYME ON THE CANINE GASTROINTESTINAL MUCOSA*

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The high concentrations of lysozyme in the stools and mucosa of chronic ulcerative colitis and regional enteritis patients have been established[3]. It has also been shown that in general the lysozyme titers and daily outputs fall with improvement[3]. The demonstration in the initial experiments of an ulcerative effect[2] of the enzyme strongly suggested that lysozyme locally initiates the lesions of regional enteritis and chronic ulcerative colitis. Additional data have shown what is statistically a probably significant increase in lysozyme activity in the gastric juice of peptic ulcer patients over normal controls[2]. The mucosa of the antrum of ulcer patients possesses definitely increased lysozyme titers when compared to similar uninvolved areas of carcinomatous stomachs[2]. This information led to the conclusion that lysozyme is probably an additional factor in the pathogenesis of peptic ulcer.

The histological demonstration of the absence of surface mucus in a Pavlov pouch and the intact stomach of a dog[2] following lysozyme perfusion indicated that the initial action is the removal of the surface mucus of the gastrointestinal mucosa, permitting secondary proteolytic digestion.

The ulcerative action of lysozyme on animal gastrointestinal mucosa has been confirmed by Wang and coworkers[7]. They, however, believe that the first demonstrable effect of lysozyme is capillary hemorrhage in the lamina propria. A histologic alteration of the intra-cellular mucus is also described by these workers. This occurred after incubation of stomach sections in lysozyme.

The following cardinal observations concerning lysozyme have been made:
1. Lysozyme occurs normally in high concentrations in tears, antral, pyloric, and duodenal mucosa, hyaline cartilage, and fetal tissues[2,4] relative to the amounts in skin, feces, saliva, urine, and blood.

2. There is a marked increase in lysozyme production in chronic ulcer-

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ative colitis, regional enteritis, and a statistically probably significant rise in peptic ulcer.

3. Lysozyme has been shown to exert a damaging effect on gastrointestinal mucosa[1,2,7].

4. Granulation tissue possesses a considerable amount of lysozyme[5].

This article reports the more recent work on the effect of lysozyme on the gastrointestinal tract. It is important to study the action of the enzyme on mucosa because the concept of its etiologic role in alimentary ulcerative disease rests in large measure upon the demonstration of an ulcerative effect.

In the study of the control sections, it was observed that the canine gastrointestinal mucosa at times exhibits sloughing of the superficial cells into the surface mucus which is apparently a normal phenomenon. It is therefore possible to find areas in normal guts where the tips of the villi are devoid of epithelium. An example is shown in Fig. 1. There were only 2 definitely pathological lesions found in all control sections. The more severe is exhibited in Fig. 2. The lesion consists of a V-shaped area of necrotic mucosa occupying less than 1 low power field. The process penetrated to a depth two-thirds the thickness of the mucosa.

TABLE 1.—RESULTS OF LYSOZYME ADMINISTRATION.

Dog	*Daily dosage in units*	*Total dosage in units*	*Duration in days*	*No. of lesions*	*No. of probable erosions*
1	14,000	840,000	60	6	1
2	27,000	459,000	17	0	3
3	1,000,000 - 3,500,000		3½	3	1
4	1,000,000—7 days				
	2,000,000—8 days	23,000,000	15	0	0

Experimental. The experimental study of the effect of lysozyme on canine gastrointestinal mucosa was undertaken employing 2 techniques: oral administration, and injection of the superior mesenteric artery.

1. Oral Administration. Crystalline egg-white lysozyme* in No. 2 gelatin capsules was administered orally to 4 dogs in this group. The dogs' weights, appearances, and behavior did not change during the experiment. At the completion of the experiment, the dogs were sacrificed with intravenous Nembutal, the gastrointestinal tracts were immediately removed, and examined grossly without washing. In this series of dogs, no hyperemia and edema comparable to that seen in the 3rd dog reported in a previous publication[2] were noted grossly. All mucosal depressions and suspicious erosions were fixed in Bouin's fluid and submitted for histologic sections. Random sections were also procured. The gastrointestinal tracts of 4 dogs sacrificed for other purposes were used for gross and histologic controls. The daily dosages, total dosage, duration of the experiment, and results varied as indicated in Table 1.

The cellular debris exhibited the characteristics of necrosis. These 2 lesions are of unknown etiology, but are distinctly different in character from those produced by lysozyme. In all instances, the erosions indicated as definite and as probable in Table 1 were qualitatively dissimilar from the preceding.

The lesions produced in the dogs fed lysozyme were characterized by a mucosal defect occupied by a mass of mucus densely packed with groups of desquamated mucosal cells retaining a viable appearance. The villi in, adjacent to, and often deep to the lesion were devoid of epithelium to a greater depth than seen in the control sections. The denudation of the lamina propria frequently extended beyond two-thirds the depth of the mucosa. Examples are shown in Figs. 3 and 4.

* Generously furnished by Dr. Lawrence Lachat of Armour and Co., Chicago, Ill.

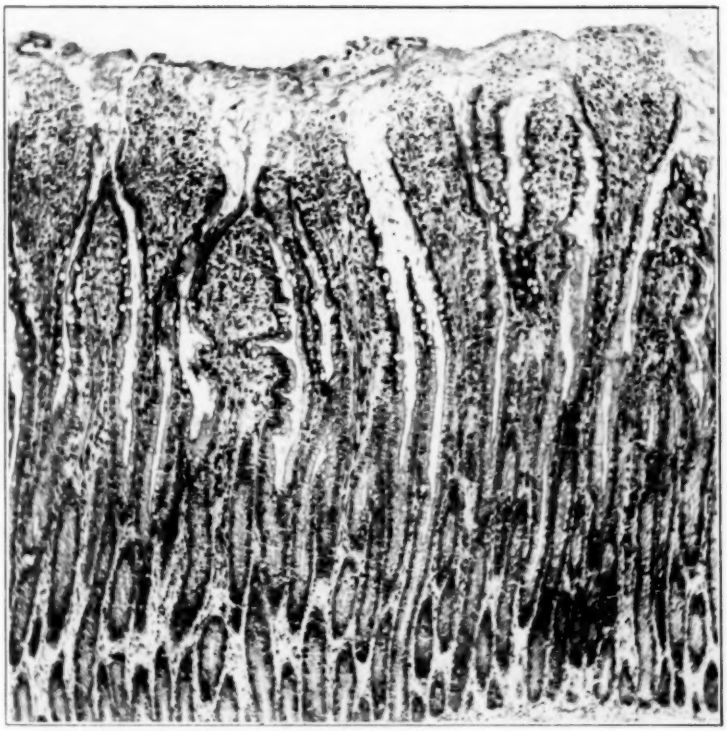

FIG. 1. Epithelial sloughing in the jejunum of a normal dog. The cells from the tips of the villi lie in the adjacent surface mucus (X 75).

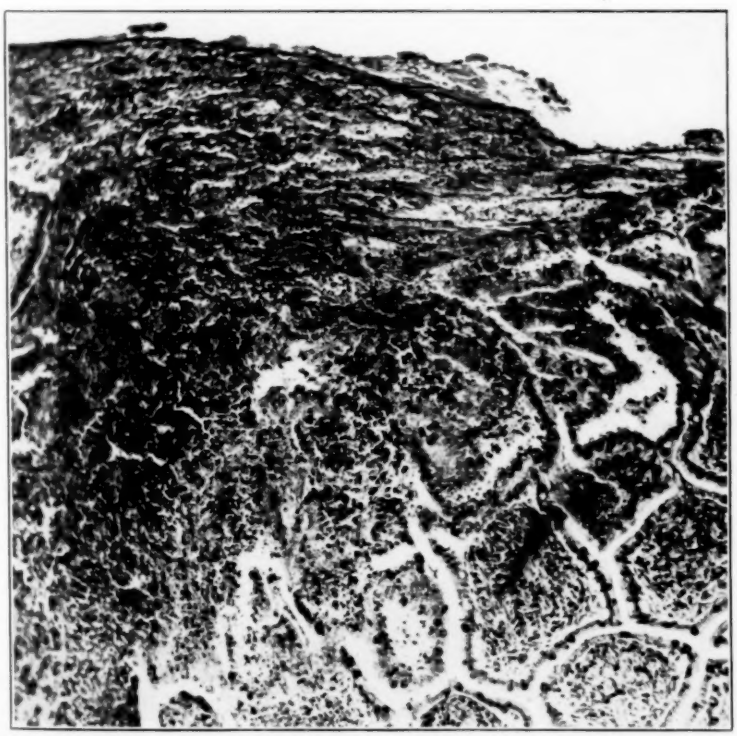

FIG. 2. A focal area of necrosis in the ileum of a control dog (X 75).

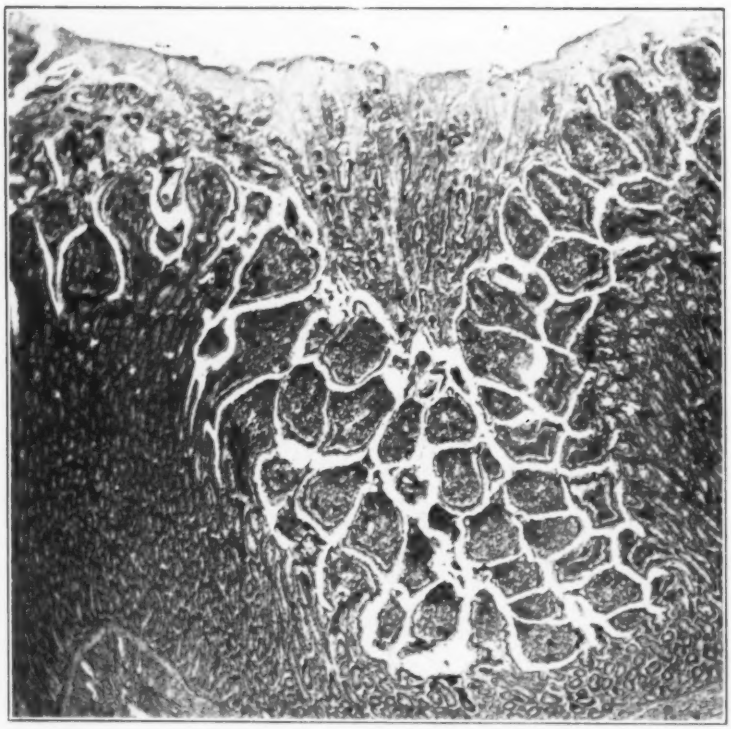

FIG. 3. Duodenal lesion in Dog 3 (lysozyme-fed). Note denudation of many underlying villi (X 44).

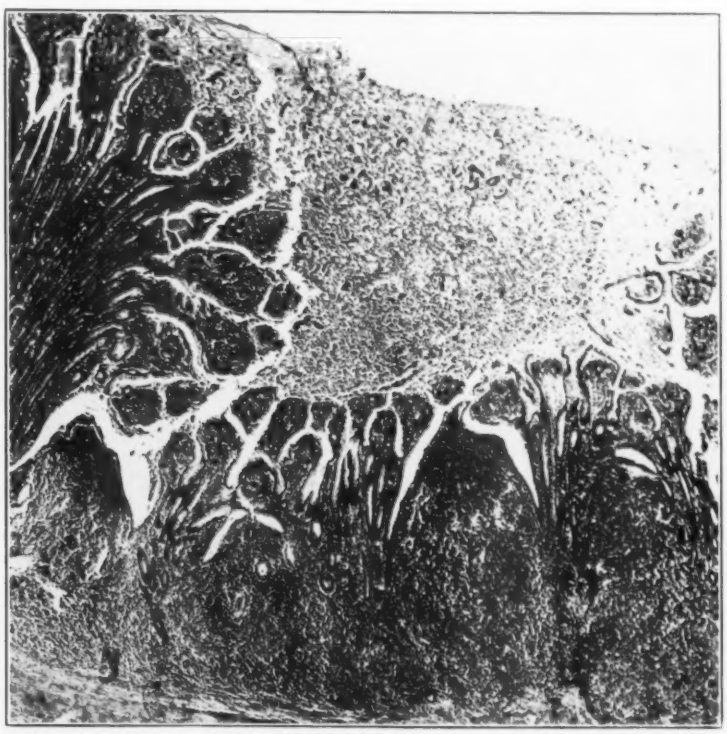

FIG. 4. Duodenal lesion in Dog 1 (lysozyme-fed) occurring at the site of a "pseudo-ulcer" (X 46).

The lesions listed as "probable" in the table were different from the "definite" ones only in extent. Compared to all control sections exhibiting superficial sloughing, they are significant.

No significant alteration in surface mucus was noted histologically in Dogs 1, 2, or 3. However, sections from Dog 4 showed only fragmentary remnants of surface mucus in the majority of sections.

mucosal collections of lymph follicles (Fig. 5). The mucosal pattern over these follicles does not retain its usual glandular and villous character; but in no instance did control sections of these areas reveal any mucosal defect or abnormal epithelial sloughing. Volini speculated on the possibility that these areas might be sites of decreased mucosal resistance. Two of the 3 definite erosions in Dog 3 were not in

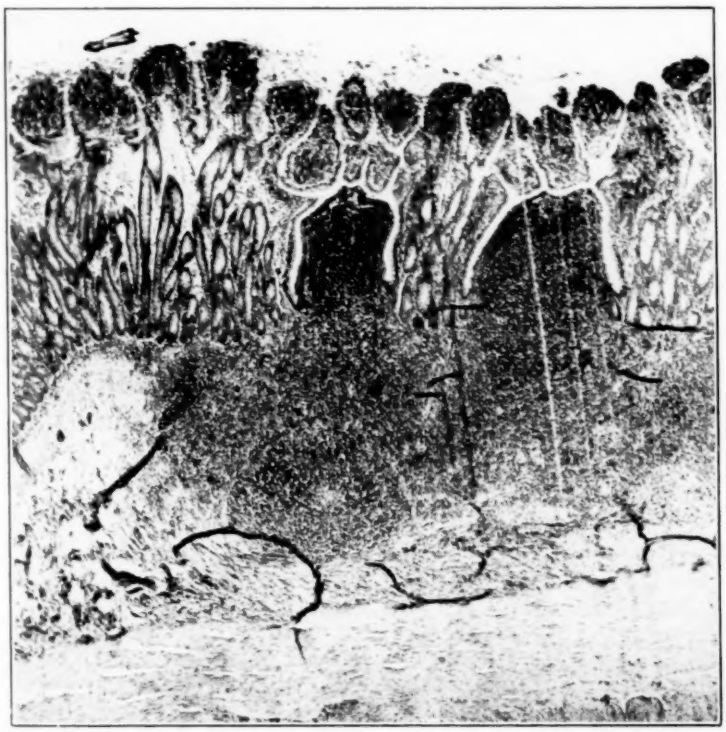

FIG. 5. View of a portion of a "pseudo-ulcer" in a control dog's jejunum (X 53).

Volini[6] called attention to circumscribed mucosal depressions occurring in the normal canine small gut; principally in the duodenum. Because of their appearance, they superficially resemble ulcerations on gross inspection; in fact, many of these sections were taken through such areas for this reason. Microscopically these areas are characterized by submucosal and association with these areas; but all 6 definite lesions in Dog 1 occurred in such sites. Of the 5 probable erosions, only 2 were so located.

These dogs bring the total number given lysozyme by mouth to 7, including those previously reported[2]. In 5 of the 7, definite lesions have been produced, and probable lesions in 6 of 7. The fact that the only failure to

produce erosions was in the dog receiving both the highest daily and total dosage is not explainable at present.

The nature of the lesions produced in these dogs suggests an acute process of short duration. For example, the erosions in Dog 1 were qualitatively similar to those in Dog 3, in spite of the extreme difference in the time element. Since many of the factors causing progressive ulcerative alimentary disease in man are absent in the dog, it is felt that progression of the lysozyme-induced lesion to classic morphologic changes is not to be expected in animals.

periment. The dogs were sacrificed 5 hours after the initial injection. Slightly different amounts were employed in the different animals. Three control dogs were used who received approximately the same doses of a solution of inactivated lysozyme. This solution was made up with 440 mg. of lysozyme in 180 cc. of isotonic saline. To this were added 20 cc. of 1 N sodium hydroxide, and the solution was placed in a boiling water bath for 30 minutes; then 20 cc. of 1 N hydrochloric acid were added to bring the solution to the original pH. This manipulation takes advantage of the destruction of

TABLE 2.—LYSOZYME INJECTION SCHEDULE.

I. Lysozyme Dog No.	*No. of Injections*	*Total time of Experiment (Hours)*	*Total dosage (mg.)*
1	3	5	176
2	4	5	320
3	3	5	360
4	3	5	360
II. Inactivated lysozyme Dog No.			*mg. of inactivated lysozyme*
5	4	5	320
6	3	5	360
7	3	5	360

2. Intra-arterial Injection. To determine the effect of the uniform interstitial distribution of lysozyme in a segment of the gut, the enzyme, in a concentration of 2 mg. per cc. (about 2860 units per cc.), was injected into the superior mesenteric artery of 4 dogs over a 5 hour period, using a 27 gauge needle and a 20 cc. syringe. For convenience it was necessary to inject slowly in a retrograde fashion at a distance of about 6 cm. from the aortic junction. It was evident that the rate of injection was not sufficient to cause an overflow into the general circulation. In every instance the mesenteric vessels in the distribution of the superior mesenteric artery were pulsating normally at the conclusion of the experiment. Slightly different amounts were employed in the different animals. Three control dogs were used who received approximately the same doses of a solution of inactivated lysozyme. This solution was made up with 440 mg. of lysozyme in 180 cc. of isotonic saline. To this were added 20 cc. of 1 N sodium hydroxide, and the solution was placed in a boiling water bath for 30 minutes; then 20 cc. of 1 N hydrochloric acid were added to bring the solution to the original pH. This manipulation takes advantage of the destruction of lysozyme in heated alkaline solution. Lysozyme assays after this treatment revealed no residual activity.

Table 2 summarizes the injection schedule, time interval, and total dosage.

The control dogs exhibited no lesions grossly, and no alteration in the normal amounts of surface mucus or in the usual vascularity of the mucosa. Microscopically, the only definite finding was normal superficial sloughing as shown in Fig. 1. Mucus could be found in the crypts in all high power fields of the control sections.

Grossly, Dogs 1 and 4 exhibited mucosal hemorrhages. In Dog 1 this was of a patchy character, primarily

in the jejunum (Fig. 6). The lesions in Dog 4 were more diffuse. No such areas were seen in Dogs 2 and 3. In comparison with the controls, the lack of surface mucus in the distribution of the superior mesenteric artery in Dogs 1 and 2 was definite, in Dog 3 was minimal, and in Dog 4 could not be appreciated due to the hemorrhagic change.

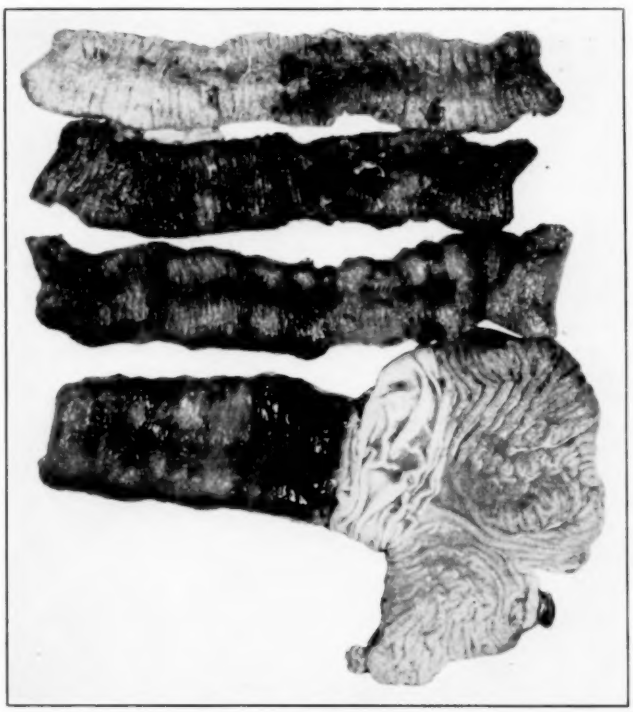

FIG. 6. Hemorrhagic lesions of the jejunum in Dog 1 (lysozyme intra-arterially).

Microscopically, all lysozyme dogs showed rather wide capillary dilatation in the lamina propria. Actual extravasation of red blood cells could be observed in all lysozyme dogs (See Fig. 7). In Dogs 1 and 4 this extravasation was apparently the source of a layer of red blood cells, serum, fibrin, and desquamated epithelial cells seen on the surface of the mucosa (Fig. 8). In some areas it was possible to trace the red blood cells from within the crypts to this surface layer, indicating that the source of the blood was from within the mucosa at the area sectioned. Fig. 9 demonstrates this. The grossly observed diminution of surface mucus was confirmed microscopically, particularly in the crypts.

Lysozyme assays were done on the small bowel mucosa of Dogs 2, 3, and 4. The results were: jejunum, Dog 2 — 40 units per gm.; jejunum, Dog 3 — 49 units per gm.; duodenum, Dog 4 — 60 units per gm.; ileum, Dog 4 — 69 units per gm. It is interesting to note that this approximates the average titers of the mucosa in regional enteritis and chronic ulcerative colitis (33 and 42.5 units per gm. respectively). Test of the serum of Dog 3 immediately before sacrifice showed a lysozyme

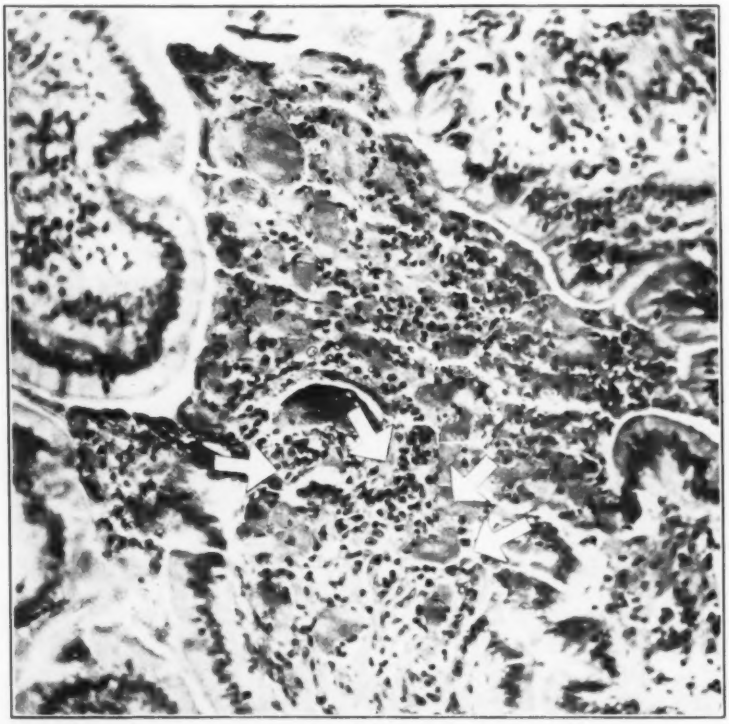

FIG. 7. Area of capillary dilatation and red blood cell extravasation in the jejunum. The arrows indicate the lamina propria of an eroded villus. This is the source of blood and cells in the overlying gland space. Dog 2 (lysozyme intra-arterially) (X 330).

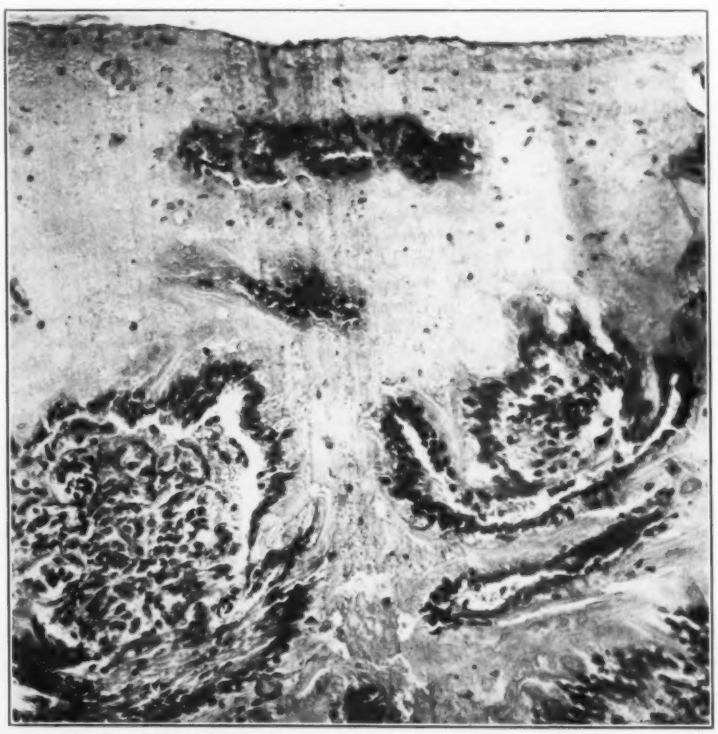

FIG. 8. Layer of blood and desquamated cells in continuity with similar material in the crypts of the jejunum of Dog 4 (lysozyme intra-arterially) (X 360).

content of 8 units per cc., in comparison with the normal of 1.5 units per cc.

Discussion. Lysozyme is regarded by us as a local initiating factor in alimentary ulcerative disease.

The recent finding[5] that the lysozyme content of granulation tissue is high raised the question of whether the high titers in colitis might be accounted for solely on this basis. This is improbable because of the finding of high stool lysozyme titers in the absence of occult blood and with sigmoidoscopically non-ulcerated mucosa. This does not imply that a portion of the stool lysozyme content may not originate from granulating surfaces when ulceration is present.

Furthermore, the proposition that lysozyme is causative rather than corollary in ulcerative alimentary disease is most strengthened by its damaging effect on gastrointestinal mucosa.

Heretofore, 3 laboratories have reported such an effect. Removal of the surface mucus with subsequent loss of superficial epithelium, hyperemia, and edema were first reported[2]. Wang and his associates[7] then emphasized capillary hemorrhage in the lamina propria. They also noted vacuolization of mucus and disorganization in the surface epithelial cells. Grace and associates[1] reported sharply circumscribed areas of inflammation and edema following the application of human tears to the mucosa of a prolapsed colostomy.

FIG. 9. The arrows indicate the continuity of red blood cells from a crypt to the surface. Jejunum of Dog 4 (lysozyme intra-arterially) (X 360).

Summary and Conclusion. By administering lysozyme orally and intra-arterially, it has been possible to duplicate the lesions reported previously, *i.e.*, removal of the surface mucus, desquamation of the epithelium, capillary dilatation and hemorrhages in the lamina propria. It still seems most probable that the observed mucosal edema and hemorrhage are secondary to the epithelial damage.

The appearance of the rectosigmoid mucosa in chronic ulcerative colitis is consistent with these observations. It is hyperemic, hemorrhagic, edematous, and lacking in surface mucus.

Lysozyme is regarded as a causative factor in alimentary ulcerative disease.

The helpful advice and suggestions of Dr. John S. Lockwood, Dr. Raymond S. Megibow, and Dr. Arthur Purdy Stout were much appreciated. Miss Joan Levison of the Department of Surgery gave competent technical assistance in the lysozyme assays and in the experimental surgical procedures.

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DIAGNOSTIC VALUE OF PULMONARY ARTERIOGRAPHY IN BRONCHIAL CARCINOMA*

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The increased incidence of bronchial carcinoma is well recognized. Established diagnostic methods, notably bronchoscopy, bronchography, and planigraphy, have been only partially successful. More recently, the cytologic study of bronchial washings has increased the accuracy of diagnosis. There remains, however, a need for other methods of investigation which will provide reliable diagnostic signs in the presence of an expanding and infiltrating neoplasm, particularly while the lesion is in an early resectable stage. It is the purpose of this study to show that certain atypical pulmonary vascular patterns are associated with bronchial carcinoma.

Injection of the pulmonary arterial tree of normal lungs with vinyl acetate demonstrates this arterial network to be rather uniform and extremely compact with the major branches closely associated with larger bronchi. A neoplasm anywhere within pulmonary parenchyma would be expected to alter this pattern to some extent.

Study of surgically extirpated pulmonary carcinomas and necropsy specimens indicates a constancy of involvement of the pulmonary arterial trunks and lesser radicles. We have noted a considerable distortion of certain segments of the pulmonary arterial tree by actual invasion or compression of blood vessels by an invading neoplasm of even small size.

The only method now available to demonstrate such vascular disturbances in the living patient is that of contrast visualization. Robb and Steinberg[2], after considerable study of the pulmonary vascular pattern in parenchymal lesions, concluded (in part): "This method gave no assistance in the diagnosis of neoplasm. It is possible that characteristic deviations from the normal vascular pattern of the lung can be worked out for pulmonary disease."

Sussman and Grishman[3] have reported that arterial occlusion in lung cancer is of considerable diagnostic importance. Recently, Neuhof *et al.*[1], have studied distortion and obstruction of the pulmonary artery and its major branches in bronchial carcinoma involving the main bronchi.

Technique. The patient is placed for a postero-anterior projection of the chest while sitting before a specially constructed multiple cassette tunnel device for rapid serial exposures. The target film distance is 6 feet. The exposure factor found satisfactory is simply an increase of 10 KV over the exposure

* Sponsored by the VA and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

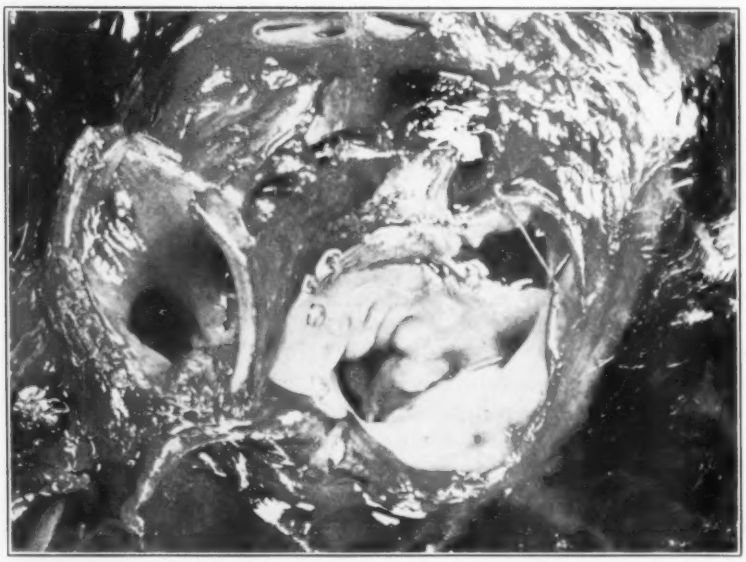

FIG. 1. Case 2. Extirpated lung, looking directly into hilus, showing carcinomatous infiltration into the left pulmonary artery.

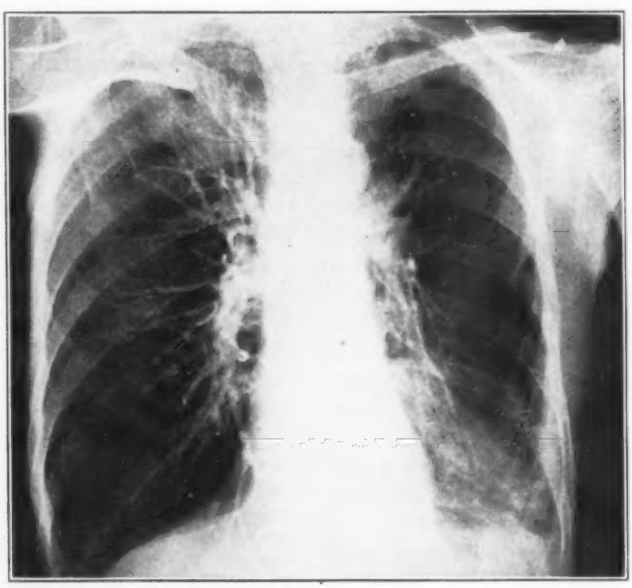

FIG. 2. Case 1. Carcinoma, left upper lobe, with emphysema and diminished vascularity. There is complete obstruction of the superior branch of the left pulmonary artery. This and subsequent roentgenograms were made after injection of diodrast.

normally used for plain film study. Fifty cc. of 70% diodrast are injected in 1 to 2 seconds through a number 13 needle into an antecubital vein. Exposures are made at 2, 4, 6, and 8 seconds after completion of the injection. The 2 and 4 second films outline the lesser circulation, and the 6 and 8 second films outline the greater circulation. No preparation of the patient has been necessary.

Results. We have performed contrast studies on cases with various types of pulmonary parenchymal disease. The vascular pattern of the pulmonary parenchyma was then scrutinized, and compared with arteriograms of patients with normal lungs.

tion of the main pulmonary trunk. One showed complete obstruction of a major part of the right pulmonary artery. The 1 case without a deviation from the normal vascular pattern had diffuse lymphangitic spread of carcinoma throughout the entire lung prior to the development of a mass in the right hilar region.

Injection of opaque media into the vascular stump of extirpated lung reveals findings comparable to those seen *in vivo*. The lung, if possible, is reinflated after removal and the main

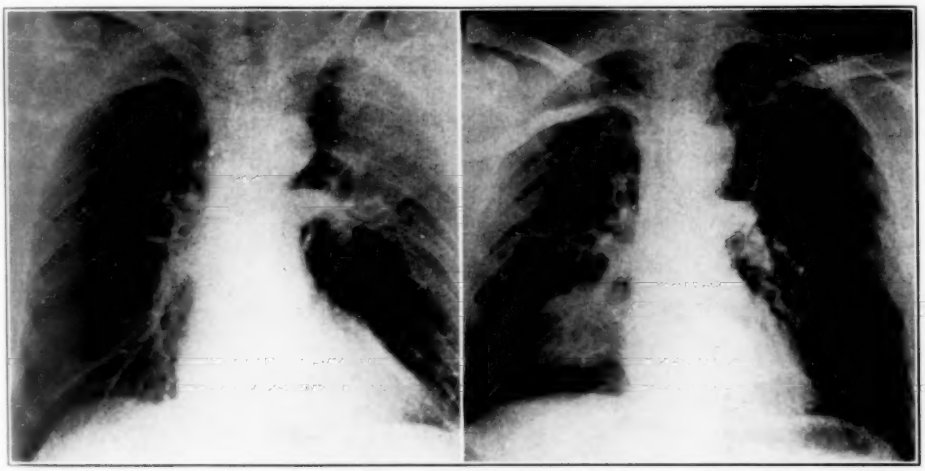

FIG. 3. Case 2. Epidermoid carcinoma, left upper lobe, with diminished vascularity of the entire left upper lobe.

FIG. 4. Case 3. Carcinoma, right lower lobe, with diminished vascularity distal to the tumor mass.

Fifteen of the patients with pulmonary parenchymal disease ultimately proved to have bronchial carcinoma. All but 1 revealed a recognizable deviation from the normal pulmonary vascular pattern. The most constant abnormality was diminished vascularity of all or part of the lung distal to the carcinoma. In addition, decreased caliber, distortion, or actual arterial occlusion of secondary radicles within the tumor mass was seen in 9 cases. Two showed displacement and distor-

bronchus occluded. The stump of the pulmonary artery is sutured, and roentgenograms are taken after injection of diodrast into the closed arterial stump. Careful comparison of the arteriogram *in vivo* is made with the injected specimen. Care is used to exclude the many artefacts possible in such a study.

In only 1 instance has the arteriogram failed to reveal diminished vascularity in the presence of bronchial carcinoma. (This was the case of lymphangitic spread.) In no instance has the pattern

of diminished vascularity been present in a purely inflammatory lesion.

There are other instances in which the vascularity of the pulmonary parenchyma is decreased. Senile emphysema, which is usually bilateral with bullous emphysema of a lung or segment of lung. Again there is ordinarily no tumefactive process present, and only rarely is the differential diagnosis of primary bronchial carcinoma entertained in one of these cases. The

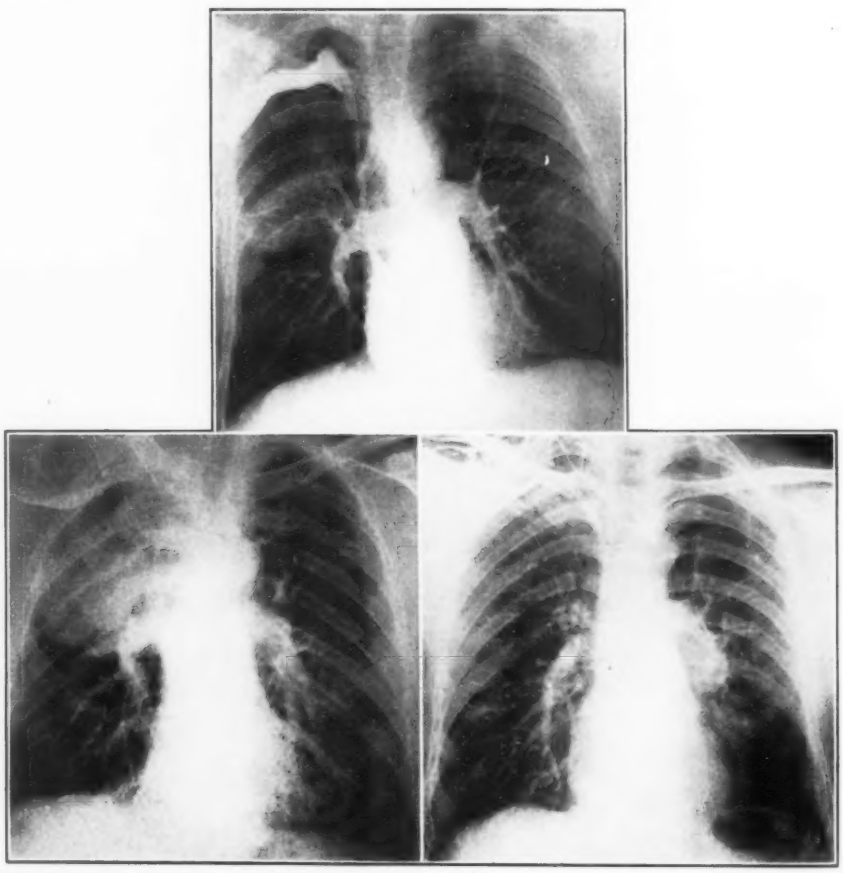

FIG. 5. Case 9. Carcinoma, right upper lobe, with diminished vascularity distal to the mass.
FIG. 6. Case 10. Carcinoma, right upper lobe, with absence of vascular markings throughout the neoplastic area, and with irregularity of the superior margin of the right pulmonary artery.
FIG. 7. Case 11. Carcinoma, left upper lobe bronchus, invading downward to involve the entire hilus and producing diminished vascularity of the entire left lung.

and not associated with a tumefactive or infiltrative neoplastic process, is associated with diminished vascularity. This diminution in the vascularity is ordinarily diffuse and symmetrical throughout both lung fields. Localized diminution in vascularity is associated only other instance of decreased vascularity is within a pulmonary abscess cavity. Within a cavity no vascular pattern is visible while the area around the abscess shows either some increase in vascularity or no change and the area distal to the abscess shows no di-

turbance of the vascular pattern. We have experienced no difficulty to date in distinguishing between a cancerous and noncancerous lesion, except in a case of massive enlargement of mediastinal nodes by tuberculosis, with compression of the superior margin of the right pulmonary artery. There was, however, normal vascularity of lung parenchyma distal to this lesion.

Discussion. We believe there are 3 reasons for the diminished vascularity occurring distal to a tumor mass in the lung. They are, in the order of importance, vascular, bronchial, and parenchymal. 1. The infiltrating neoplasm may compress or obstruct primary or secondary arterial branches. 2. Partial bronchial obstruction with resultant segmental or lobar obstructive emphysema produces diminished vascularity. 3. Necrosis of lung parenchyma causes complete avascularity of the involved portion of lung. One or more of these factors in combination may be present in any individual case.

In our experience diminished vascularity in early carcinoma has been the result of vascular occlusion. This appears to be a constant finding, no matter where the neoplasm is located. We are aware of the diminished vascularity incident to obstructive emphysema alone.

TABLE 1.—ANGIOGRAMS AND LESIONS FOUND IN BRONCHIAL CARCINOMA.

Case	*Angiogram*	*Gross Pathology*	*Microscopic*
1–HMG	Obstruction, sup. branch left pul. art., with dimin. vascularity LUL	Not available (died elsewhere with cerebral metastases)	Not available
2–OET	Obstruction, sec. radicles, with dimin. vascularity, LUL	Mass involving major portion LUL, invading upper lobe art.; origin LUL bronchus	Epidermoid carcinoma
3–LCA	Decreased calibre of vessels within and dimin. vascularity distal to mass	Mass involving mid. lobe, app. originating from a sec. bronchus	Adenocarcinoma
4–IB	Pattern unaltered by lymphangitic spread of neoplasm	Diffuse invasion, rt. lung; origin rt. main stem bronchus	Poorly differentiated carcinoma
5–CEJ	Avascularity of entire rt. lung	Mass encircling RMSB with loculated cavities in neoplasm replacing lung parenchyma; origin RMB	Epidermoid, poorly differentiated
6–LJC	Displacement of sec. radicles and dimin. vascularity distal to mass	Origin RUL bronchus with extension inferiorly	Carcinoma, undifferentiated
7–LFH	Dimin. vascularity, entire rt. lung	Origin RLL bronchus with infiltration RLL	Undifferentiated CA
8–WLB	Dimin. vascularity, entire rt. lung	Origin rt. eparterial bronchus with obstruction of rt. pul. art. by encircling mass	Epidermoid, poorly differentiated
9–SM	Dimin. calibre of vessels adjacent to mass and dimin. vascularity distally	Nodular mass, 5 cm. diam., RUL, extending peripherally	Epidermoid, well differentiated
10–GB	Irregularity, sup. margin, rt. pul. art.; obstruction, sec. branches, rt.; dimin. vascularity, RUL	Not available. Diag. proved by exploratory thoracotomy and biopsy elsewhere	Not available
11–JEJ	Decreased calibre of sec. branches, lt. pul. art., dimin. vascularity, entire lung	Origin LUL with mass extending downward to involve entire hilus	Epidermoid carcinoma
12–TAW	Dimin. vascularity of RUL	Origin RUL bronchus with infiltration entire RUL and mediastinum	Epidermoid carcinoma
13–SCD	Decreased vascularity, LUL, distal to tumor mass	Origin LUL bronchus. Localized to LUL	Adenocarcinoma
14–FKO	Decreased vascularity, RUL, with complete obstruction of upper portion of rt. pul. art.	Origin RUL bronchus with extension to mediastinum	Undifferentiated carcinoma
15–RLS	Dimin. vascularity, entire rt. lung	Mass involving entire rt. hilus and mediastinum	Undifferentiated carcinoma

Summary. 1. The results of pulmonary arteriography in 15 cases of bronchial carcinoma have been presented.

2. Diminished vascularity of the portion of involved lung distal to the tumor mass was found in 14 cases. Other changes were encountered less often. One patient with diffuse lymphangitic spread of neoplasm revealed no discernible abnormality.

3. These changes, in our opinion, are explained by obstruction of arterial branches, bronchial obstruction with resultant emphysema, and necrosis of pulmonary parenchyma.

4. Other changes encountered less frequently include decreased caliber of vessels with displacement, distortion and obstruction of main pulmonary artery.

5. Diminished vascularity was also found to occur in non-neoplastic diseases, notably senile pulmonary emphysema, bullous emphysema, and lung abscess. However, these conditions are rarely associated with a tumefactive process and we have encountered no difficulty in distinguishing the pattern from that produced by carcinoma.

Conclusion. We believe pulmonary arteriography should be added to the diagnostic armamentarium in cases of bronchial carcinoma.

The pulmonary vascular pattern shows, in nearly all cases, characteristic deviations from the normal.

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THE SIGNIFICANCE OF BILIRUBIN PARTITION IN HEPATO-BILIARY DISEASES*

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The diagnostic significance of the partition of the serum bilirubin into a direct and indirect fraction in jaundice, exclusive of the hemolytic form, is not clearly established. An investigation of this question on a large number of jaundiced patients with verified diagnoses, therefore, appeared indicated. In addition, the recently available, extensive material of liver biopsy specimens offered a chance to study the relation of this partition to liver cell damage. Moreover, since the pathogenesis of jaundice has been associated with changes of the bilirubin fractions, information gained from such studies may serve in re-evaluating this as yet obscure mechanism.

It has long been known that in performing the qualitative diazo reaction of van den Bergh in some instances of jaundice, alcohol had to be added before the characteristic red color was seen (indirect bilirubin or bilirubin A), while in others this color appeared without the addition of alcohol (direct bilirubin or bilirubin B)[25]. It was assumed that bilirubin which gave the indirect reaction had not reached the liver cells and that which gave the direct reaction had already passed through them and had regurgitated from the bile capillaries[6,18,19]. The chemical or physical differences between the 2 forms of bilirubin have been the subject of much controversy. Apparently they differ mainly in their colloidal state which in turn is responsible for variations in the protein binding[2].

Many methods have been described for the quantitative determination of both bilirubin fractions in the blood[9,17,20,22,24]. The application of photoelectric colorimetry to the van den Bergh reaction[10], particularly as modified by Ducci and Watson[3], has found widespread acceptance.

* Supported by grants from the Dr. Jerome D. Solomon Memorial Research Foundation and the Committee on Scientific Research of the American Medical Association.

After the addition of diazo reagent to serum, the red color may appear promptly and then gradually deepen on standing up to 30 minutes or it may appear only after several minutes. This gave rise to several descriptive terms such as prompt direct, delayed direct, biphasic, and others. Watson demonstated that there are only 2 components to the reaction, a prompt one and a delayed one, the latter being apparently due to gradual coupling of the indirect bilirubin[26]. Therefore, quantitatively, the direct bilirubin was considered that amount which had reacted in 1 minute (as determined by the Malloy and Evelyn method). This fraction and the total serum bilirubin are considered the only values of practical significance[3,26]. Because the reaction is stopped in 1 minute, Watson prefers the term prompt-reacting bilirubin, which will be employed here.

Using this sensitive photochemical method, it was found that bilirubin is present in normal human serum up to 1.20 mg. per 100 cc., less than 0.2 mg. per 100 cc. of which reacted promptly[26].

Material and Methods. In 279 jaundiced patients, 580 determinations of prompt-reacting and total serum bilirubin were performed according to Ducci and Watson[3]. Of these, 233 determinations were performed in 114 patients with cirrhosis, 228 in 81 patients with acute hepatitis (viral or toxic), and 123 in 84 patients with extrahepatic biliary obstruction. The diagnoses in these patients had been established by clinical and laboratory observations including a series of hepatic tests[15] and also by the follow-up course, liver biopsy or autopsy. Only patients with a total serum bilirubin of 2.0 mg. per 100 cc. or more were included. Too few cases of hemolytic jaundice were available for statistical evaluation. In addition, 31 normal adults were used as controls.

Liver biopsy specimens were obtained 153 times in 132 patients, in most cases, by aspiration with a Turkel needle[23] using the right lateral approach through the 9th interspace. In a few cases the liver specimen was obtained at laparotomy. In 34 instances of this series, the biopsy specimen was obtained at a time when the jaundice had subsided. The biopsy specimens were fixed in Carnoy's solution and stained with hematoxylin and eosin or Mallory's aniline blue. The presence of minimal or marked liver cell damage was determined without knowledge of clinical laboratory findings according to the criteria established by Popper, Steigmann and Szanto[16].

T values were used as a test of significance for the differences between the means of the bilirubin determinations in the various forms of jaundice. These values were obtained from the following formula[12]:

$$T = \frac{M_1 - M_2}{\sqrt{\sigma_{M_1} + \sigma_{M_2}}}$$

where M_1 and M_2 are the respective means and σ_{M_1} and σ_{M_2} are the standard errors of these means. This latter value was obtained by dividing the standard deviation of the mean (σ) by the square root of the number of determinations (N) less one:

$$\sigma_M = \frac{\sigma}{\sqrt{N-1}}$$

T values, also used as a test of significance of the differences between the number of cases with minimal and with marked liver cell damage as related to the levels of the prompt-reacting and total serum bilirubin and the prompt/total bilirubin ratios, were obtained from the following formula[12]:

$$T = \frac{\%_{(A)} - \%_{(B)} - 0}{\sigma(\%_{(A)} - \%_{(B)})}$$

where $\%_{(A)}$ is the percentage of cases with marked liver cell damage with the bilirubin fraction under consideration above a given level and $\%_{(B)}$ is the percentage of cases with minimal liver cell damage with the bilirubin fraction under consideration above the same given level. $\sigma(\%_{(A)} - \%_{(B)})$ is the standard deviation of the percentage differences and is obtained by the following formula:

$$\sigma(\%_{(A)} - \%_{(B)}) = 100\sqrt{\frac{P_{AqA}}{N_A} + \frac{P_{BqB}}{N_B}}$$

where p is the proportion above the given level, q the proportion below and N the total number of cases. A T value above 2.00 is taken here as showing significant correlation.

The degree of correlation (T) was found using Dunlap's biserial correlation formula[4]:

$$r_{biserial} = \left(\frac{M_h - M_t}{\sigma_t}\right)\left(\frac{P_h}{y_h}\right)$$

where M_h represents the mean of the bilirubin fraction under consideration of the case

with significant liver cell damage and M_t the mean of this fraction in all the cases. σ_t is the standard deviation of this latter mean, P_h the proportion of the total cases in the group with marked liver cell damage and y_h the value of the ordinate on a normal curve at the point that divides the total distribution into two parts, with the proportion of the area above the point equal to P_h. Correlation coefficients range from plus to minus one with zero indicating no correlation at all. For the size and distribution of the sample used in these calculations, an r value above 0.25 may be considered as significant. This value will result in a T value of 2.00 when tested by the null hypothesis[12]. An r value above 0.31 will yield a T value of 2.50 or more.

Results. A. DIAGNOSTIC VALUE OF THE RATIO BETWEEN PROMPT-REACTING AND TOTAL BILIRUBIN. The evaluation of the diagnostic value of the van den Bergh reaction, the initial values only were considered (Table 1). On the average, patients with extrahepatic biliary obstruction had the highest concentration of both total and prompt-reacting bilirubin, while those with cirrhosis had the lowest values. The ratios of the prompt-reacting to the total bilirubin did not show this characteristic difference, although in general the ratios in jaundice were considerably higher than in normals. The ratios were similar in cirrhosis and infectious hepatitis on the one hand, and in toxic hepatitis and obstructive jaundice on the other.

The significance of the difference between the means for the prompt-reacting and the total bilirubin and the prompt/total bilirubin ratio was statistically expressed by T values (Table 2). Some of the differences in the bilirubin concentrations mentioned were statistically significant; such as the difference in the total bilirubin between cirrhosis and the other forms of jaundice, and in the prompt reacting bilirubin, between biliary obstruction and

TABLE 1. MEAN PROMPT-REACTING AND TOTAL SERUM BILIRUBIN AND PROMPT/TOTAL BILIRUBIN RATIOS IN 279 JAUNDICED PATIENTS AND 31 NORMALS*

Diagnosis	Number of Cases	Mean Prompt Reacting Bilirubin (in mg./100 cc.)	Mean Total Bilirubin (in mg./100 cc.)	Prompt/Total Bilirubin Ratio (in %)
Normals	31	0.15	0.51	29.41
Cirrhosis	114	4.34	9.90	38.64
Infectious Hepatitis	44	5.32	14.58	38.96
Toxic Hepatitis	37	6.86	15.43	43.48
Extrahepatic Biliary Obstruction	84	8.16	18.72	43.45

* Initial determinations only.

TABLE 2. T VALUES FOR DIFFERENCES IN THE MEANS OF THE PROMPT-REACTING AND TOTAL SERUM BILIRUBIN AND THE PROMPT/TOTAL BILIRUBIN RATIO BETWEEN THE VARIOUS ETIOLOGIC FORMS OF JAUNDICE*

	Prompt Reacting Bilirubin	Total Bilirubin	Prompt/Total Bilirubin Ratio
Biliary Obstruction—Cirrhosis	4.67	5.60	2.69
Biliary Obstruction—Infectious Hepatitis	3.20	1.96	1.73
Biliary Obstruction—Toxic Hepatitis	0.96	1.15	0.01
Infectious Hepatitis—Cirrhosis	1.33	2.88	0.12
Toxic Hepatitis—Cirrhosis	2.00	2.06	1.79
Toxic Hepatitis—Infectious Hepatitis	1.18	0.28	1.40

* A T value above 2.00 indicates a significant difference.

cirrhosis or infectious hepatitis. However, there was no significant difference between biliary obstruction and toxic hepatitis, or between toxic and infectious hepatitis. The only significant difference in the prompt/total bilirubin ratio was found between extrahepatic biliary obstruction and cirrhosis.

The prompt/total serum bilirubin ratios showed a similar distribution in each of the various etiologic forms of jaundice (with the exception of the hemolytic variety) with almost half the values between 40 and 50% (Fig. 1).

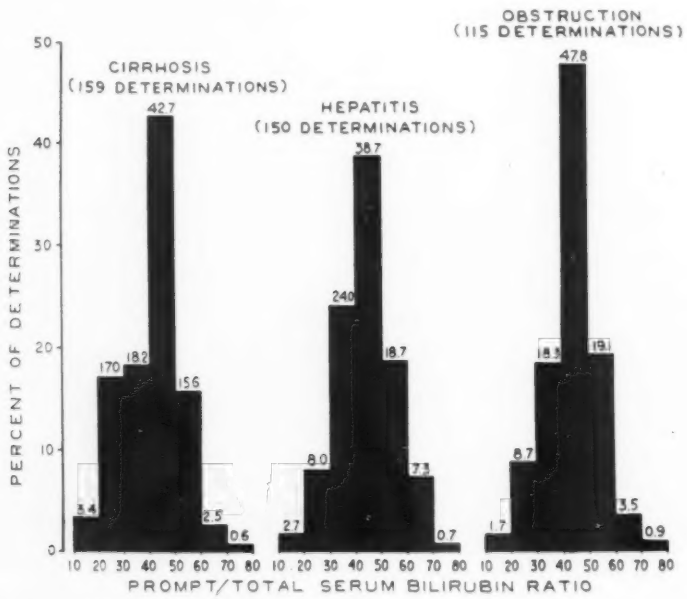

FIG. 1. Percentage distribution of the prompt/total bilirubin ratio (expressed in %) in cirrhosis with jaundice, acute viral and toxic hepatitis and extrahepatic biliary obstruction.

However, when plotted against the total serum bilirubin, the ratio between the prompt-reacting and total bilirubin ranged between 0 and 80% when the total bilirubin was below 10 mg. per 100 cc., with most of the values below 40% (Fig. 2). When the total bilirubin was above this, four-fifths of the values were between 30 and 50%. As the level of the bilirubin rose, the ratio tended to fall. When the total serum bilirubin was above 40 mg. per 100 cc., the prompt/total ratio was again below 40% in two-thirds of these cases.

In 14 cases there was evidence of intrahepatic biliary obstruction[21] which persisted longer than 3 days. Eight had toxic hepatitis; 4 infectious hepatitis; and 2 cirrhosis. The ratio of the prompt-reacting to the total bilirubin varied between 25.7 and 53.0% with a mean value of 40.9%, a figure which does not differ from that of the non-obstructive group.

In 50 cases, the serum bilirubin values were followed until one or both fractions returned to normal (Table 3). In slightly less than half, the total bilirubin became normal first, while the prompt-reacting fraction remained above 0.3 mg. per 100 cc. At this time one-third of them showed persistent signs of hepatic damage (malaise, anorexia, tenderness of the liver and ascites

TABLE 3. CLINICAL AND LABORATORY EVIDENCE OF PERSISTENT ACTIVITY IN 50 CASES OF JAUNDICE FOLLOWED UNTIL ONE OR BOTH BILIRUBIN FRACTIONS RETURNED TO NORMAL

Prompt-Reacting Bilirubin	Normal	Still Elevated	Normal
Total Bilirubin	Still Elevated	Normal	Normal
Number of Cases	9	23	18
Percent with Persistent Clinical Findings	11	35	17
Percent with Persistent Laboratory Findings	33	70	50

in the cirrhosis group) and more than two-thirds showed abnormal findings in 2 or more of the following hepatic tests: cephalin flocculation, thymol turbidity, albumin/globulin ratio, cholesterol ester ratio, serum alkaline phosphatase, urinary urobilinogen determination and sedimentation rate. In the cases in which both fractions because normal simultaneously, persistent clinical findings and abnormal results in the hepatic tests were less often found. In the few instances in which the prompt-reacting bilirubin became

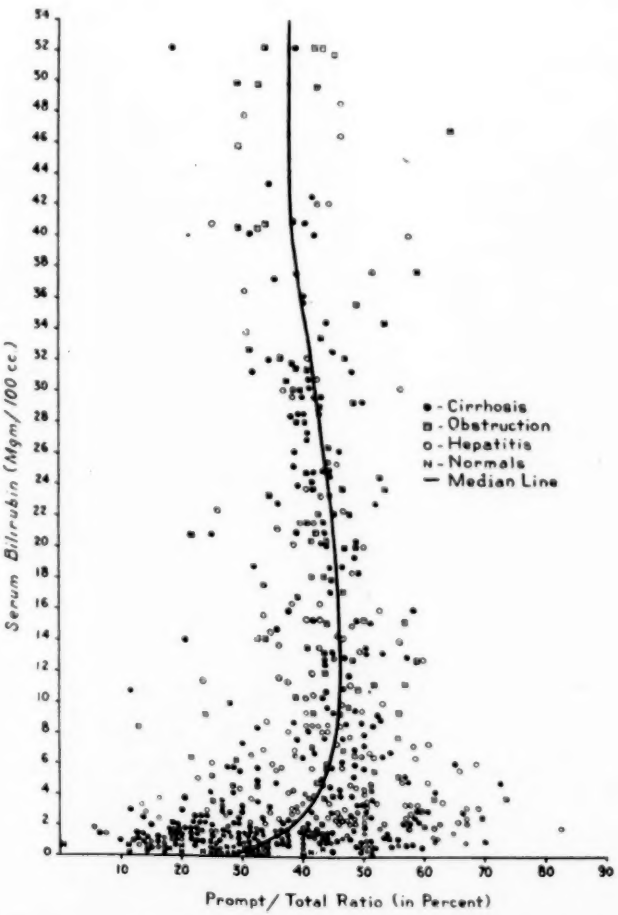

FIG. 2. Correlation between total serum bilirubin and the prompt/total bilirubin ratios in normals and patients with various hepato-biliary diseases.

TABLE 4. CORRELATION OF LEVELS OF THE BILIRUBIN FRACTIONS IN PATIENTS WITH MINIMAL OR MARKED MORPHOLOGIC LIVER CELL DAMAGE*

No. of Cases with Liver Cell Damage	Total Serum Bilirubin						Prompt-Reacting Bilirubin						Prompt/Total Bilirubin Ratio 45%	
	2 mg./100 cc.		8 mg./100 cc.		10 mg./100 cc.		4 mg./100 cc.		6 mg./100 cc.		12 mg./100 cc.			
	Below	Above	Below	Above	Below	Above	Below	Above	Below	Above	Below	Above	Below	Above
Minimal	8	30	16	14	18	12	18	12	23	7	21	9	13	17
Marked	26	89	33	56	35	51	39	50	50	89	43	46	36	53
T Value	0.19		1.44		1.65		1.54		2.26		2.22		0.29	

* A T value above 2.00 indicates significant correlation.

normal first (less than 0.3 mg. per 100 cc.) persistence of signs of clinical activity and abnormal results in the hepatic tests were even less commonly encountered.

B. CORRELATION OF THE BILIRUBIN FRACTIONS WITH THE PRESENCE OF MORPHOLOGIC LIVER CELL DAMAGE. A comparison of the histologic picture of liver biopsy specimens (some of them obtained at a time when jaundice had subsided) revealed no relation between the presence or absence of jaundice and the degree of liver cell damage (Table 4). However, when a sufficiently high level of the total bilirubin was chosen as a borderline (12 mg. per 100 cc.), the association between bilirubinemia and liver cell damage became statistically significant. The same was true for the prompt-reacting bilirubin where the borderline was about 6 mg. per 100 cc. However, there was no borderline of the prompt/total bilirubin ratio which permitted the establishment of a significant difference between groups with minimal or marked liver cell damage. These findings were further reflected in the correlation coefficient between the bilirubin fractions and the presence of minimal or marked morphologic liver cell damage. Significant correlations, although not of a high degree, were found between both the prompt-reacting and the total bilirubin and morphologic liver cell damage, the former being somewhat more significant (Table 5). No significant correlation was found between the presence of liver cell damage and the prompt/total bilirubin ratio.

Discussion. Differences in the total and prompt-reacting bilirubin are of little differential diagnostic value. Only in cirrhosis are both less elevated on the average than in the other conditions. They reflect the stage of the condition rather than the etiology of the jaundice. In patients with manifes

jaundice (total serum bilirubin above 2.0 mg. per 100 cc.), the ratio between prompt-reacting and indirect bilirubin and consequently the ratio between the prompt-reacting and indirect bilirubin is not significantly different in the various hepato-biliary diseases. In a large series of cases there is a great deal of overlapping of the ratios which may not be apparent in smaller groups from which the assumption stems that such a ratio may be significant[8]. Although this study concerns itself with quantitative values, the same conclusions can be considered for the results of the qualitative van den Bergh reaction.

persistent elevation of the prompt-reacting fraction above 0.3 mg. per 100 cc. generally indicates persistent activity of the disease process; whereas, return of the prompt-reacting fraction to normal even with slight elevation of the total bilirubin generally indicates subsidence of the disease. This is in agreement with the previous findings of Capps and Barker[1], the former having suggested the investigation of this particular relation in this study.

The most significant change in the serum bilirubin fractions between normal serum and serum from a jaundiced patient (exclusive of hemolytic jaundice) is that the ratio of

TABLE 5. DEGREE OF CORRELATION BETWEEN TOTAL SERUM BILIRUBIN, PROMPT-REACTING BILIRUBIN, PROMPT/TOTAL BILIRUBIN RATIO AND THE PRESENCE OF MARKED MORPHOLOGIC LIVER CELL DAMAGE*

	Mean Value of Cases with Minimal Liver Cell Damage	Mean Value of Cases with Marked Liver Cell Damage	Correlation Coefficient (r)
Prompt-Reacting Bilirubin in mg./100 cc.	4.48	6.32	0.310
Total Bilirubin in mg./100 cc.	11.22	16.24	0.272
Prompt/Total Bilirubin Ratio in %	42.94	41.10	−0.104

* An r value above 0.25 is significant.

This conclusion is also supported by the lack of correlation between morphologic liver cell damage and the ratio between the prompt-reacting and total bilirubin in contrast to the correlations found between liver cell damage and the absolute values of the prompt-reacting and total bilirubin[16].

This study does in no way deny the value of the ratio in hemolytic jaundice nor does it militate against the diagnostic importance of the elevation of the prompt-reacting bilirubin before that of the total in slight or incipient jaundice, as has been found in early infectious hepatitis[22]. In manifest jaundice, the bilirubin partition into prompt-reacting and indirect forms is of no value if hemolytic factors are excluded. In defervescent jaundice, a prompt-reacting to total bilirubin is increased. As jaundice becomes very deep, the ratio tends to decrease, although not to the level of non-jaundiced sera. These changes occur similarly in both parenchymal and obstructive jaundice. Differences in the prompt-total bilirubin ratio are, therefore, related to the level of the total bilirubin rather than to the disease.

These findings call for a re-evaluation of the present concept of the pathogenesis of jaundice. Since the relation of the bilirubin fractions to each other is identical in parenchymal and obstructive jaundice, there is no reason to assume a difference in the mechanism of the bilirubinemia in these 2 conditions. Previously, these findings might have been explained by

regurgitation through breaks in the liver cell cords in obstructive jaundice and through damaged liver cells in parenchymal jaundice[6]. However, interruptions in the continuity of the liver cell cords are not necessarily demonstrable in obstructive jaundice in biopsy specimens[13]. In fact, histology offers no clues as to the mechanism of jaundice. The liver cell cords are usually intact in liver biopsy specimens in both obstructive and parenchymal jaundice, although in autopsy specimens the cords may be broken up in both instances[13]. Also the fact that once jaundice is present, the indirect bilirubin rise keeps pace with the rise of the prompt-reacting fraction, and in high degrees of jaundice may even exceed it, is difficult to reconcile with simple regurgitation. Furthermore, the presence of prompt-reacting bilirubin in normal serum cannot be adequately explained by the older concept of bile pigment metabolism.

In considering another explanation, it should be remembered that an important role is ascribed to the Kupffer cells in mammalian bile pigment metabolism[5,11]. The role of the Kupffer cell is probably 3-fold. In addition to the formation of some indirect bilirubin, it picks up indirect bilirubin formed elsewhere by the reticuloendothelial cells, chiefly in the spleen and bone marrow, and finally transmits it to the liver cell for excretion. In this process, the bilirubin is changed to the prompt-reacting type, as suggested by Watson[26]. The amount of prompt-reacting bilirubin present in normal serum suggests that in the transmission from Kupffer to liver cell, some of the bilirubin escapes into the bloodstream, either directly or via the lymphatics[7].

Early in parenchymal jaundice or extrahepatic biliary obstruction, the liver cells are unable to accept prompt-reacting bilirubin from the Kupffer cells because of impairment of either liver cell function or excretion. Therefore, the prompt-reacting bilirubin formed is returned to the bloodstream. Difficulty in the transmission of bilirubin from the Kupffer cells to the liver cells causes its accumulation in the Kupffer cells, which is seen histologically. This, in turn, interferes with their ability to remove indirect bilirubin from the bloodstream and the level of this fraction also rises, but to a lesser degree than that of the prompt-reacting fraction. As jaundice becomes deeper, both the prompt-reacting and the total bilirubin rise in almost parallel fashion because the Kupffer cells take up and transform only a proportional amount of the increased indirect bilirubin. In severe degrees of jaundice, the capacity of the Kupffer cells to pick up and transform indirect bilirubin is probably exceeded (or possibly diminished due to accumulated bile pigment), and thus there is a decrease in the prompt/total bilirubin ratio. These considerations obviously do not exclude the possibility that regurgitation through the cholangioles, for example, may influence bilirubin levels also[27].

It may finally be pointed out that in obstructive jaundice and toxic hepatitis, the means of the prompt/total bilirubin ratios are higher than in infectious hepatitis. This is not due to difference in the degree of jaundice, as may be the case with the difference between biliary obstruction and cirrhosis. Therefore, the mesenchymal (inflammatory) reaction, which is marked in infectious hepatitis and cirrhosis when compared to toxic hepatitis and biliary obstruction[14], is most likely responsible for this difference, since it entails increased Kupffer cell activity.

Summary. The prompt-reacting and total serum bilirubin and the prompt/total bilirubin ratio were determined repeatedly in 279 patients with jaundice, exclusive of the hemolytic variety, and in 31 controls. Mean

values were calculated for cirrhosis, infectious hepatitis, toxic hepatitis and extrahepatic biliary obstruction. The absolute values of the bilirubin fractions and the ratio between them are of no value in differentiating medical and surgical jaundice. However, elevation of the prompt-reacting fraction in subsiding jaundice indicates persistence of the disease process.

The amount of prompt-reacting serum bilirubin was found to depend on the degree of jaundice. As jaundice develops, the prompt-reacting fraction increases more than the indirect. When jaundice is established, both fractions rise fairly parallel. When the bilirubinemia exceeds 40 mg. per 100 cc., the increase of the indirect fraction is greater than that of the prompt-reacting one.

The presence of liver cell damage, as seen in 153 liver biopsy specimens, showed no correlation with the presence or absence of jaundice or the prompt/total bilirubin ratio. A slight but statistically significant correlation was found between the presence of liver cell damage and the absolute values of the prompt-reacting and total bilirubin.

These findings agree with the hypothesis that bilirubin is changed from the indirect to the prompt-reacting from in the Kupffer cells and then transmitted to the liver cells for excretion. Some of the prompt-reacting bilirubin, not taken up by the liver cells, accounts for the small amounts normally present in the blood. In parenchymal jaundice, the liver cells are unable to accept all of the bilirubin and in obstructive jaundice they are unable to excrete it; in either case, resulting in a return of prompt-reacting bilirubin to the bloodstream and the accumulation of bilirubin in the Kupffer cells. This accumulation impairs bilirubin uptake and subsequently the indirect bilirubin increases.

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DISODIUM HYDROGEN PHOSPHATE THERAPY IN LEAD POISONING

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It has been well established that lead is perhaps the most typical of the cumulative poisons[8]. Following its absorption via any of several possible routes (mouth, respiratory tract, skin or conjunctiva), lead establishes depots in the bones and the soft tissues. The dense bone and the subepiphyseal zones of the bone are the body areas of greatest accumulation, while of the soft tissues, the liver and kidney show the largest amounts of stored lead[4,5]. The laws governing the deposition of lead in these sites and its release from them are similar to those for calcium release and deposition. Therefore, acidosis, iodides, parathyroid hormone and other agents which remove calcium from the depot sites, also remove lead. This parallelism creates a continuous potential danger inherent in excessive amounts of lead in the depot areas, because accidental or incidental mobilization of lead is credited with the periodic acute exacerbations of symptoms in chronic plumbism, sometimes precipitated by dietary factors and most commonly by the acidosis created by intercurrent infections (such as pneumonia and others), surgical operations or alcoholism. Because of the periodic exposure of persons suffering with chronic plumbism to these dangers it has become the duty of the physician and the toxicologist to establish effective therapy for those who have become victims of the lead hazard. Many therapeutic measures have been tried in attempting to cure the disease.

A survey of the more important contributions to this field revealed 3 distinct methods of approach: *a*, Deposition of lead in its depot sites without subsequent deleading[10]; *b*, deposition of lead in its depot areas with subsequent carefully managed acidosis or iodide mobilization from these sites[1,2,3,9]; and *c*, by safe deleading without previous therapeutic deposition[4,5]. Consideration of the methods shows that the first gives only symptomatic relief, and the second has the same inherent dangers found in accidental mobilization. This turned our attention to the important contributions of Gant[4,5]. His work, which was mainly involved with careful establishment of body lead levels in normal and in lead-intoxicated persons, using irreproachable analytical technique, also included what we felt to be the most rational route to the therapy of plumbism.

Basing his theory on the finding of Shelling[6], who showed that low-calcium high-phosphorus diets were successful in deleading in the rat, and that leaded animals improved much more on disodium hydrogen phosphate than on calcium carbonate, Gant proposed the use of disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$) as a therapeutic agent for plumbism. In

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expanding this theory, Gant advanced the following advantages of sodium dibasic phosphate: "*a*, It will precipitate lead in its most insoluble form, the tertiary phosphate ($Pb_3(PO_4)_2$), and apparently this occurs to a large extent *in vivo*. *b*, Absorbed lead, and that which is unabsorbed in the gastro intestinal tract, is therefore largely detoxified. *c*, The slight increase in pH (alkalosis) may mobilize some lead previously deposited in the bones. *d*, Excess sodium ions disturb the calcium balance, calcium and lead being drawn from the trabeculae. *e*, Mobilization and detoxification occur simultaneously. *f*, Free ionized lead is diminished by a depression of ionization by the phosphate which is known to be an excellent buffer. *g*, The salt is also a good cathartic, thus eliminating the use of others."

With these postulated advantages in mind we undertook a study designed to evaluate this form of therapy on a clinical basis.

Technique Employed. All patients included in this study had clinical symptoms, physical findings or history pointing toward a diagnosis of lead poisoning. These clinical manifestations were also checked by determination of the urinary excretion of lead present in a 24-hour period, using as a criterion for diagnosis of lead exposure the ranges set up by Gant: below 80 micrograms per liter (80 μgm./L), normal; 80 to 150 μgm./L, abnormal exposure; above 150 μgm./L, definite toxicity when accompanied by clinical symptoms. The determinations were made polarographically by one of the authors, the usual precautions against possible contamination having been rigidly observed in the collection of the samples analyzed. Following this confirmation of the diagnosis of lead intoxication, therapy was instituted with disodium hydrogen phosphate heptahydrate, given by either the intravenous or oral route, normal dietary calcium being maintained to guard against hypocalcemia. The course of the patient's progress was followed closely clinically and, in some cases, by repeat analyses of 24-hour urine specimens during the course of the medication. In all cases a polarographic determination on the 24-hour urine specimen was performed at the end of the course of therapy.

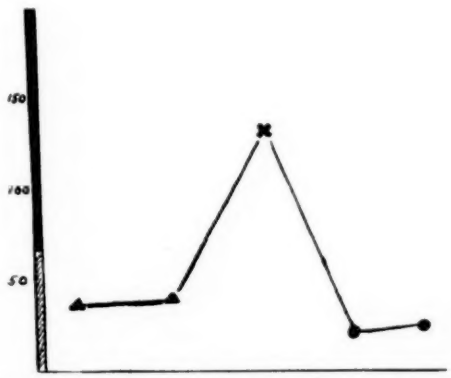

FIG. 1. Deleading effect of disodium hydrogen phosphate on a control. (Ordinate in terms of 24-hour urinary lead levels expressed in micrograms of lead per liter, the shaded area showing the usual limits of normal excretion.)

■ = diagnostic samples, no medication
× = sample taken second day of medication with 15 grains Na_2HPO_4 t.i.d.
● = samples taken 3d and 4th day after cessation of medication

Results. *A. Patients with lead intoxication.*

Case Reports. CASE 1. A 27-year old male complained of anorexia, severe abdominal cramps, and constipation of 3 weeks' duration. He had worked in a smelting works as a furnace tender for the last 3 years. A

Burtonian line was noted on his gums. Thirty-three stippled cells were found in the blood smears and a 24-hour urine specimen revealed the presence of 500 μgm. of lead per liter. Therapy consisted of 60 grains of sodium dibasic phosphate 3 times daily by mouth for 15 days. Symptoms disappeared shortly after therapy was started. The 24-hour urine specimen obtained at the end of therapy showed a lead level of 152 μgm./L.

Case 2. A 52-year old male complained of anorexia, nausea, constipation, abdominal cramps and loss of muscular strength for 2 weeks. He was a painter by trade. Many basophilic stippled cells were found. The 24-hour urine specimen revealed the presence of 526 μgm. of lead per liter. Therapy consisted of 60 grains of sodium dibasic phosphate orally 3 times a day for 1 week. Symptoms had completely disappeared, though the 24-hour urine lead level was 215μgm./L; therefore treatment was resumed for another week, at the end of which the level had dropped to 138 μgm./L.

Case 3. A 29-year old male complained of anorexia and constipation of 1 month's duration. He had been a general laborer in a smelting works for the last 2 years. Examination of the blood smear showed no basophilic stippling but did show 1.3% reticulocytes. A 24-hour urine specimen revealed the presence of 464 μgm. of lead per liter. Ten grains of sodium dibasic phosphate were given intravenously 3 times a day for 1 week. The 24-hour urine lead level dropped from 464 μgm./L to 170 μgm./L. The patient was asymptomatic at this time.

Case 4. A 45-year old male, who had worked as a brass and aluminum grinder but who had been exposed to the fumes of melted lead in the foundry in which he worked, complained of severe abdominal cramping pains, anorexia and constipation for 1 week. A lead line was noted. A moderate normochromic anemia and 63 basophilic stippled cells were present. Five grains of sodium dibasic phosphate were given 3 times a day intravenously for 5 days. The pain disappeared the 2nd day and bowel movements returned to normal on the 4th day. The 24-hour urine lead level dropped from 158 μgm./L to 58 μgm./L.

Case 5. A 67-year old housewife complained of headaches, dizziness, "spots before her eyes," anorexia, abdominal cramps and right wrist drop of 2 months' duration. No lead line was evident, due to adentia. A moderate normochromic anemia and 6 basophilic stippled cells were present. Five grains of sodium dibasic phosphate 3 times a day intravenously were given for 5 days. The symptoms disappeared but the wrist drop persisted. The lead level dropped from 158.4 μgm./L before therapy to 73 μgm./L after therapy.

Case 6. A 24-year old male, who had worked for 6 months as a spray painter, complained of anorexia, nausea, vomiting, abdominal cramps, headache and muscular weakness of 2 weeks' duration. A Burtonian line was present and 21 stippled cells were found in the blood smear. Therapy consisted of 30 grains of sodium dibasic phosphate 3 times daily for 5 days by mouth. His symptoms disappeared by the 3rd day. The lead levels were as follows: 190 μgm./L before therapy, 432 μgm. on the 2nd day of therapy, 162 μgm. on the 4th day of therapy and 100 μgm. 5 days after treatment was completed.

Case 7. A 48-year old male, a professional spray painter for 10 years, complained of anorexia, nausea, and severe abdominal cramps for one week. No Burtonian line was evident, but a few stippled cells were present. Treatment consisted of 60 grains of sodium dibasic phosphate 3 times per day orally for 5 days. The patient was asymptomatic by the 4th day. Lead levels were 163 μgm./L before therapy, 774 μgm. on the 4th day of therapy, and 124 μgm. after treatment was completed.

Case 8. A 42-year old female had worked at ceramic decorating which involved the use of lead pigment paints for 12 years. She complained of anorexia, dizziness, constipation and peripheral paraesthesias for 1 month. No Burtonian line was present. Fifteen grains of sodium dibasic phosphate were administered 3 times daily by mouth for 7 days. The patient was asymptomatic after the 4th day of therapy. Lead levels were 128 μgm./L before therapy, 159 μgm. the 2nd day of treatment, and 98 μgm. after the treatment was completed.

We were impressed with the value of sodium dibasic phosphate in completely alleviating the clinical symptoms in the above cases of lead intoxication. Neither albumin nor abnormal microscopic findings were present in the urine during the course of therapy in the above cases. The definite drop of the 24-hour urine lead levels from the toxic range to a level below that considered toxic was demonstrated in every case. However, in order to establish an actual "deleading effect"

3 control cases were studied and more frequent determinations performed during the course of sodium dibasic phosphate administration.

B. Controls.

CASE 1. One of the authors who was completely asymptomatic had an original urinary lead level of 87.2 μgm./L. He had been a spray painter while working his way through medical school 7 years previous to this determination. Fifteen grains of sodium dibasic phosphate 3 times per day orally were administered for 5 days. The lead levels were as follows: during the 1st day of therapy, 152.3 μgm./L; the third day of therapy, 45.8 μgm.; and on the 5th day after cessation of therapy, 38.2 μgm. No symptoms were noted at any time during the course of the experiment. Urinalysis remained negative.

CASE 2. The wife of the above author was also asymptomatic and had an original urine lead level of 17.5 μgm./L. Medication was the same as in the above case. On the 1st day of therapy the lead level was 48.9 μgm./L, on the 3rd day of therapy, 38.2 μgm.; but 5 days after treatment the lead level was 10.1 μgm. Again no symptoms were noted and the urinalysis remained negative.

CASE 3. The other author, also asymptomatic, had an original lead level of 31 μgm./L. Treatment was the same as in the above 2 cases. On the 2nd day of treatment the level was 135.6 μgm./L and 4 days after treatment was 25 μgm. Urinalysis again remained negative.

The results of the 3 control cases appear to demonstrate adequately: 1, that sodium dibasic phosphate is nontoxic; 2, that it markedly increases the excretion of lead in the urine (deleading); and 3, that during the deleading process with markedly increased lead excretion no symptoms were produced.

Comment. We realize that this study consisted of a relatively small series of cases, but we believe that the results obtained give adequate basis for the belief that sodium dibasic phosphate has definite value in the treatment of plumbism and merits more extensive application and investigation. The simplicity of administration, the low cost and the removal of active and potential dangers concurrent with lead intoxication by an actual deleading effect (see graph) while alleviating symptoms safely, are advantages which make it appear superior to any other form of therapy thus far available. In acute cases it may be given intravenously as a 5% solution in 5- to 10-grain doses 3 times a day. Chronic cases may be treated with 15 grains 3 times per day orally for a minimum of 5 days. Exact dosage has not yet been determined, but amounts as high as 60 grains orally 3 times a day have been given with safety.

Summary. 1. Eight cases of lead intoxication have been presented which were treated with sodium dibasic phosphate in doses varying from 15 to 60 grains 3 times a day orally, or 5 to 10 grains 3 times a day intravenously.

2. Complete cessation of symptoms was noted in every case and the 24-hour urine lead level was brought to a non-toxic range.

3. Sodium dibasic phosphate is nontoxic and acts as an effective deleading agent.

4. We believe that this form of therapy merits further investigation.

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UNDER THE CHARGE OF

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THE EPIDEMIOLOGIC METHOD APPLIED TO NUTRITION

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THE range of interest within epidemiology is today much greater than the single concern with behavior of communicable diseases as they affect groups of people or populations. To include other kinds of mass or crowd disease was a logical step in the transition by which the group manifestations of infections themselves came to be understood as something a good deal more than the catastrophic or unusual events recognized as epidemics. If knowledge was to be had as to why epidemics came into being and why they subsided, the whole natural history of these processes had to be understood, sporadic and endemic occurrence as well as epidemics. The epidemiologic method was applied in turn to infections of plants and of animal species other than man, because the circumstances commonly stood in relation to the morbid processes of human beings. If communicable disease so evidently conformed to biologic laws, if these processes could be interpreted in terms of medical ecology[44], then other diseases of population groups presumably could be approached in similar fashion. Gradually, it becomes apparent that the epidemiologic method is applicable to most mass diseases of man, and to injuries as well, to the extent that they represent problems of population groups.

Some diseases as problems of groups

of people are matters of relatively recent interest, although long studied clinically and by experimental methods. Observations on trench foot[43a] and accidents[43b] are examples of an epidemiology of trauma, and a start has been made in the difficult field of neuropsychiatric disorders. Other mass diseases of non-communicable nature have been more thoroughly explored. As long ago as 1924 Haven Emerson[33] wrote of diabetes as an epidemiologic phenomenon, and the contributions of Joslin[60], and Joslin, Dublin and Marks[61] are continued in the recent work of Wilkerson[135]. Chapin recognized an epidemiology of cancer in 1928[20], to which Greenwood[46] and others made early contributions, and Lombard[74] and Levin[72] and Ryle and Russell[111] are currently active. Of diseases other than the infections, none have been studied by field methods to greater extent than the varied conditions included under nutrition. The origins of modern field epidemiology are commonly placed with the observations of Panum on measles[95], Budd on typhoid fever[14] and Snow on cholera[122]. That is fair enough so far as communicable diseases are concerned, but Goldberger[42] with his studies on pellagra was surely the Panum of nutrition when he found the causes and traced the course of that disease in the homes of people.

The Epidemiologic Method. Workers in the communicable diseases have long recognized 3 basic approaches to knowledge of causation and behavior, the ultimate objective being an improved prevention and control and a better clinical management. Information is to be sought from bedside study of the sick individual. The controlled conditions provided by laboratory experiment are a principal reliance of modern medicine. The third general method is epidemiological, through observation of disease as it occurs under natural conditions in whole populations. The "patient" in this latter circumstance is a group of persons, sometimes many thousands, and includes both the sick and the well. Comparisons are made of the frequency and kind of disease manifested by different fractions of that population, from such simple divisions as those of age, race and sex, to the more complicated separations by occupation, social status, economic situation and many others, all in terms of the time and place that disease occurs. The usefulness of the epidemiologic method is not so well recognized in other fields as in the communicable diseases, nor is practice so general. It is to be understood that the epidemiologic method by no means is suggested as a substitute either for clinical study, which is clearly the point of departure in any investigation of disease, or for the experimental method. However, clinical study may reach a point of diminishing returns, and experiment may be restricted by the limitations of experimental conditions. This third method, the epidemiologic approach, has many times supplemented the other two; and has to its independent credit some of the outstanding discoveries in communicable and other disease. It is an addition rather than a substitute for other methods of scientific inquiry.

More general use of the epidemiologic method is inhibited as much as anything by a common interpretation of its content as limited to the recognition of incidence and prevalence, and sometimes even those 2 features are not clearly differentiated. Together they make up an elementary epidemiology as fundamental as the determination of temperature, pulse and respiration in clinical medicine. A second larger objective is to use those data in the derivation of cause; wherein cause relates both to origin and behavior. The theory

of multiple causation is taken as principle, namely that cause has to do with factors within the host and the influences of environment as well as with the characteristics of a direct inciting agent. A third feature of epidemiologic procedure is the design of experimental field studies, sometimes to test the validity of laboratory observations, more commonly in search of further evidence to support hypotheses of cause as derived from naturally occurring disease. The close analogy with clinical methods is evident. Experimental procedure is also incorporated.

The epidemiologic method is characterized principally by the unit of observation, which is a population, while in clinical medicine it is a single individual. To be sure, clinical and experimental techniques also employ multiples of persons, but as a summation of individual observations, and the discipline by which they are examined is medical statistics. In epidemiology the group is considered as a whole, and observations relate to the whole, where some members are healthy and some are diseased. The methods further contrast in that one is deductive and the other inductive. The clinician can rarely be certain whether or not his patient of child bearing age will have a baby during the coming year, granting the absence of pregancy at the start. Not only is the epidemiologist assured that his patient will have that experience, but he can estimate with considerable accuracy the number of babies. Some of the patients of the clinician will be diabetic and some will not, but the epidemiologist, at least in American practice, will expect all of his communities to be so, but only to the extent of about 1.3%[135]. It is to the potentialities and accomplishments of the epidemiologic method in nutrition and with nutritional disease that further discussion is directed.

Nutrition a Fundamental Part of the Ecologic Complex. The group problems of human nutrition would appear especially suited to an epidemiologic approach. They are clearly ecologic, in the sense that ecology is concerned with the balance of forces resulting from interaction of man with his environment, and epidemiology has been defined as medical ecology[29], that part of ecology which deals with health and disease.

The lines of dependency for food are called food chains[133]. Each species, including man, is a link in many such chains, to the extent that man stands at the top of a pyramid of food chains that reach to a broad base which is the soil, since only plants are able to synthesize food directly from the raw materials of the earth and the air. Because of his complex diet man becomes a link in countless food chains, a part of a highly organized structure, where functioning depends on cooperation and competition of all the diverse links. The simpler conditions of a primitive existence are complicated by a modern civilization where huge urban areas produce no part of their food supply, and there is a world population that has increased from 900,000,000 towards the end of the eighteenth century to some 2,300,000,000 today[97]. Years ago, in 1798, the gloomy curate Malthus[78] predicted that eventually the population of the world would outstrip its food supply. It has always had difficulties. From 1200 to 1600 A.D. England had a famine about every 15 years[15], while in China from 108 B.C. to 1911 A.D., 1,823 such events are recorded in not far from that number of years[98]. The Malthusian concept has not operated conspicuously in western Europe[54], largely because of the expansion of commerce, a developing technology and the discovery of empty continents, but considered from a long

term and a world standpoint the concept has done rather well.

Most problems of health and disease may be viewed either in relation to the individual or to the community. The group relationship has a special significance in nutrition because the causes of many disorders are as much sociological as physiological. A number of considerations suggest the usefulness of the epidemiologic approach. More often than in most fields, the need exists to test by field trial the results of experimental studies from the nutrition laboratory. Again, assessment of the effect of nutrition on populations by direct experimental attack is largely impractical because of the life span of man, and the long years of observation required. As a consequence, the sampling techniques and the short term observations of the epidemiologic method are substituted. It often happens that the observed physical signs of a nutritional disorder in a population are not alone due to a simple dietary deficiency, but are strongly influenced and sometimes primarily determined by the presence of other disease. An observed deficiency may be the result of an existing difficulty, but it may also be no more than the residue of one no longer present. Conditions furthermore change, sometimes within brief periods and for no clearly evident reason, so that periodic field surveys with evaluation of currently existing conditions are essential to sound administrative practice.

Epidemiology and Nutrition. The original correlation between intake of food and disease promptly directed attention to the groupings of cases that occurred in wars, on shipboard, in asylums and prisons, and in the more ordinary pursuits of man. This was the beginning of an epidemiology of nutritional diseases which, for such conditions as scurvy, is as old and well established as that of smallpox. The original concern was of the spectacular epidemics of the time, which rivalled those of infectious origin to such extent as to engender belief that they were indeed communicable processes. Progressively, clinical observation of patients was extended to other family members, principally as a means for better understanding of the individual illness. Scurvy had its epidemiological highlights in the work of Lind[73] in the British Navy (1752) and of Barlow[8] in the pediatric clinics of London (1883). When biochemical research eventually defined ascorbic acid deficiency as the direct cause of the disease, a new precision was brought to field studies through improved methods of measurement, and control measures profited by the refinements which characterize modern field practice.

The history of the other vitamin deficiencies is much the same, with epidemiologic activities finding a principal development in the work of Goldberger[42] in pellagra, Mori[87] for xerophthalmia, and Takaki[131] in beriberi. The British contributions to rickets are an early example among all mass disease of a thoroughgoing approach to etiology in terms of multiple causation. The observations of Ferguson[36], those of the British Commission in Vienna of 1923[80] and the studies of Mann[79] illustrate field investigations that employed clinical and laboratory methods in wholly modern fashion, and additionally gave weight to social as well as physiological and environmental causes.

Field studies have been used to equal extent in appraising population deficiencies in minerals. The classical example is of iodine, with the original observations in France[21] and in Switzerland[129] finding further application in defining the areas of endemic goitre in Great Britain[92a,94] and in this[67] and other countries[65,93]. It is without purpose to list all substances studied, from

cobalt[132] in diseases of sheep to iron[86] in man, except to emphasize the current interest that centers around the element fluorine as it bears on dental caries[35] and mottled enamel[3,59]. The work in this dental field is one of the better illustrations of epidemiology turned to other purposes than the communicable diseases.

The interests just presented deal with specific nutritional disorders associated with increased or decreased amounts of a particular nutrient, of such extent that the change marks a definite departure from the values that correspond to the physiological state. Under all circumstances, an understanding of the normal, which is health, is essential to an interpretation of disease. The two are not absolute and opposite conditions, but relative states, each with gradations of varying moment, and they merge one into the other. Many impairments short of absolute disease are embraced within health[71d] and disease ranges from inconsequential disorders to fatal illness. If disease is any state short of health, then health must be defined, whether the situation is that of disease of a person or of a population. Epidemiology necessarily has given much consideration to a definition of the normal as related to its unit of study, which is populations of people. An epidemiology of health[82] in contrast to the epidemiology of disease is recognized. In this development, the field of nutrition has had a leading part through surveys of populations having as their objective a definition of the normal nutritional state and the conditions necessary for the basic maintenance of health. This base that is health or normality, this band of physiological variations that stands for a state of well-being, when defined within reasonable and logical limits, is an essential adjunct in appraising the departures from that value. Such departures may be conceived as projecting vertically from that base, spike-like as a series of individual specific nutritional diseases, and either upward or downward, as something is added or withdrawn from the ecologic complex that represents normality. With equal ease the deviations may be conceived as broad general movements from the established base, disregarding the individual components of specific nutritional disease; thus upward as obesity and downward as inanition.

A third general use of the epidemiological method is in evaluation of the effect of one mass disease in conditioning the reaction of a host population to a second. Such relationships are clearly recognized in clinical study of the individual patient; to cite examples, the effect of diabetes on susceptibility to infection, of whooping cough as a precursor of tuberculosis, or calcium deficiency and the frequency of fractures. Experimental nutrition is directing much effort towards determining the effect of varying grades of nutritional inadequacy and of nutritional disease on such conditions as heart disease, hypertension, diabetes, cancer and many others. The epidemiological method has been less used to this end and yet it would appear to offer special promise in such complicated problems as cirrhosis of the liver, and in assessment of malnutrition as a factor in host resistance or susceptibility to a variety of diseases. This is the horizontal approach in epidemiology, as contrasted to the vertical direction of effort within the pattern of a single selected disease.

Criteria for Distinguishing Health and Disease of Populations. Accepting the premise that distinction between health and disease is rarely clear cut, that degrees of normality and abnormality exist, and that the two situations tend to shade into each other, their separation is seen to involve two con-

siderations so far as it relates to epidemiologic needs and the study of population groups. The first is a proper identification of the specific abnormal events occurring within a population group; and secondly that these numbers of events be related to a recognized standard deemed usual. The first consideration is common to clinical medicine and to epidemiology. It presents varying degrees of difficulty according to the pathological condition at hand.

In the traumatic disability that follows accidental injury, the distinction between normal and abnormal is relatively simple, and yet even here arbitrary decision has to be made on a basis of extent of clinical effect or days lost from normal activities. With many diseases the concept of normal is more complicated, more indefinite, and a fair appraisal consequently has greater weight in the value attached to the ultimate conclusion. With health judged according to structure, function and physicochemical characteristics of the body, 4 general methods are available by which to determine that quality.

The first is complete conformity with some fixed set of standards, biochemical, physical or otherwise, as determined arbitrarily by competent authority. The value of this method is limited by the competence of the authority and the soundness of his data; also by the rigidity of the system which is scarcely biological. An alternative criterion is that of clinical reaction, that the individual is well and not handicapped by some disturbance manifested by measurable symptoms. Mild indispositions are recognized as lying within the limits of normality. A third approach may be termed the arbitrary statistical system, based on deviation from an established normal by a specified amount, in respect to such characteristics as height, weight, and amounts of hemoglobin. The non-arbitrary statistical system acknowledges no pre-determined judgment, is based on the normal distribution curve and accepts a shifting standard of normality according to differences in time and place. Those methods listed as second and fourth are believed to serve best the purposes of epidemiologic inquiry in nutritional disturbances, and presumably clinical needs as well. Under any circumstance the standard adopted is to be set forth clearly, for it affects results and interpretation in important degree.

The second consideration in respect to a designation of normal is met by initially computing rates of frequency of the observed abnormal events for the population under study. That population may be the total persons within the selected universe, or children within that population, or such unusual fractions as those suffering from cancer or engaged in a particular occupation. The significance of the observed rates is then judged through comparison with an acknowledged standard, a control. This standard may be found within the group observations themselves, through comparing one fraction with another; it may be the existing frequency in a population judged to express an ideal or satisfactory situation; it may represent an average of the observed population over representative periods; or it may correspond to a common level, based on the known total behavior of the disease as it affects man everywhere that adequately recorded data exist. The decision as to what is to be taken as a population normal clearly rests on the nature of the question for which an answer is sought and the design of the experiment to obtain that answer. The matter will have further attention when field techniques are considered.

Disease, like health, is subject to wide variations in nature and in behavior within what is clearly a single entity.

This is true of most morbid processes. The range is often from the mildest, indefinite response to situations highly exacting in death and disability. Sometimes described as the spectrum of disease, the term biologic gradient of disease is believed to express more appropriately the series of variations which together form the composite that characterizes a morbid process. Conditions in nature represent anything but the static situation implied by the word spectrum; but rather a shifting relationship, qualitatively and quantitatively changing as the biologic forces which give rise to them also change; which is to say, with the varying totality of causation. Diphtheria infection is a familiar example of the biologic gradient of disease, where the total experience within a population includes a large component of latent or subclinical infection, considerable numbers of a disease form that is little more than simple tonsillitis, a lesser frequency of massive involvement of the structures of the upper respiratory tract, sometimes circumscribed laryngitis or tracheitis, to the ultimate in reaction which is fulminating hemorrhagic diphtheria of rapid evolution and highly fatal course. Some of these clinical responses differ so greatly from the usual as to suggest an independent disease entity, but all are part of a single process.

Definition of the biologic gradient, whatever the disease under consideration, is an essential part of epidemiologic analysis. Through quantitating the factors active in one form of the disease, and comparing them with those of another, information is often to be had of the causes that act in the total process. The presence of a factor in the inconsequential forms of a disease and its absence in malignant manifestations may be the key to a vital factor in causation.

For some diseases a biologic gradient can be constructed with relative ease. For nutritional diseases associated with lack of adequate amounts of a particular nutrient, 4 divisions[22] serve satisfactorily. They correspond 1, to a state of unsaturation of tissues but of such degree as to produce no functional impairment; 2, a potential deficiency approaching pathologic levels; 3, latent deficiency disease, of minor degree and short of clear clinical assessment; and finally 4, manifest deficiency disease. The problem of a gradient is not so simple with such nutritional disorders as obesity[90], or the opposite situation, i.e., inanition[1,5], but the need is as great and there is equal promise of epidemiological return. The construction of the gradient is more than a matter of including those slight abnormalities to be recognized through methods for earlier or improved diagnosis, because the range of disease processes commonly extends into changes never perceptible by clinical methods. For their recognition biochemical and other procedures are necessary, as in diphtheria, where immunological and serological techniques alone define the important part of the gradient represented by latent infection.

Multiple Causation of Mass Disease. Writing recently on the etiology of the deficiency diseases of nutrition, Kruse[71d] brings out the paramount position occupied by diet, stating that since 1910 the prevailing doctrine has been faulty diet as the sole cause. The situation has a parallel in the attitude towards infection that existed a quarter century ago, and to an extent still prevails. In the flush of fresh discovery, the infectious agent dominated all thought in infection, and in turn the vitamins and the other nutrients in nutrition.

As Bayne-Jones[10] and many others have stated for infection and Kruse[71d]

for nutrition, a realization gradually took form that disease was no longer being studied, but rather parts of a disease; that too frequently the parts were taken to be the principal phenomenon; that in pursuit of knowledge about the agent of disease, the main objective was being lost. In both fields the belief was voiced of a need to return to first principles, to study disease instead of its parts and to search also for cause in factors that lie in other directions than the agent which directly starts the process.

Increasingly, infections have come to be interpreted as ecologic phenomena[41], the result of an interaction between a direct agent of disease, an infected host and the environment in which both live. Cause as it relates to the origin and course of communicable disease in population groups has been demonstrated to rest regularly in all three elements, although in varying degree according to individual circumstances, and often with one or other dominant. From this it follows that similar disease processes may arise from dissimilar causes, of which the agent is but one. Winslow[30] expressed this when he spoke of the tubercle bacillus as not *the* cause of tuberculosis, but one of the causes. As other mass diseases were submitted to epidemiologic analysis, the same general plan for an identification of cause was found to apply. Reason suggests that nutritional disorders of groups of people can be interpreted to advantage according to the same holistic pattern, where three elements form the ecologic complex from which disease results.

The recognized agents of disease include substances of physical, chemical or biologic nature; in nutrition, principally chemical. Their effect is sometimes through excess amounts and sometimes through a deficiency, with the result that disease is to be recognized as either a positive or a negative phenomenon[38].

The host factor contributes to causation first through inherent characteristics which are variously anatomic or physiologic, genetic or developmental, in respect to age, race, sex and others. The special susceptibility associated with the period of growth[55] is outstanding. Acquired host characteristics come into play through metabolic and anatomic changes from other disease processes, that range from the infections to neoplasms, from psychosomatic and other mental disease to include even fractures. More general biochemical and physical adaptations of people considered as groups are called forth by the impact of environment, of which the current aging of populations is an increasingly significant host factor.

For the reason that problems of nutrition and of food supplies are so intimately a part of the broader ecologic system that governs the life and welfare of man, it is understandable that environment[71c,114] has an important part in the genesis of nutritional disorders. The practical approach to analysis of environment factors in disease causation is furthered by separating its many features into 3 broad categories[44]: the physical environment, the biologic and the social, the latter having to do with those features originating in the association of man with his fellow man.

For practical application in the field, the scheme presented is enlarged to advantage through appropriate subdivisions but this simpler form serves to demonstrate principle. The idea of an ecologic concept of mass disease, holistic in nature, is not new in this or other fields. Roussel[108] recognized in 1866 that the conditions required for production of nutritional disease were both dietary and non-dietary, an idea not unexpected, for that was the period that man first began to think ecologi-

cally[47]. McCarrison[75] furthered these views in 1921 with detailed consideration of host factors that were both inherent and acquired, and of environment in respect to its physical, biological and social components. It is in essence the viewpoint developed by Platt[101], Kruse[71d] and a number of others in publications of the day.

To understand disease as the resultant of ecologic forces within a dynamic system made up of an agent of disease, a host and an environment, is to simplify the definition of cause. Under varying circumstances an outbreak of nutritional disease may be due to a true deficiency of the diet or it may occur in the presence of an adequate diet which the host is unable to assimilate and utilize because of a peculiar constitution arising from other endemic disease. By contrast the chief factor in causation may be a biologic factor of the environment, an epidemic among plants or animals, with a resulting effect on the food supply. It may be principally related to the social environment; commonly an economic influence, or in relation to crowding, housing or inadequate transport. Each problem of nutritional disease in a community tends to be individual. Potentially all 3 elements of host, agent and environment are concerned in causation, commonly one more than the others, but rarely any one alone. To distinguish causes, conditions and factors and to differentiate contributing, inciting and precipitating influences, appears unnecessary complication.

Many parts enter into the fabric of cause and they are of decidedly different nature. They have relative weight that must be quantitated. So quantitated, the independent variables are isolated and assessed in relation to the observed total effect. This approach would seem to have promise in equalizing the emphasis so long placed on dietary influences, on the agent of disease. It should aid in integrating nutritional disorders into the general medical and social interests of communities, rather than to consider them as things individual and set apart. The specific epidemiologic interests to which attention is now directed are presented from this point of view.

Epidemiologic Method in Practice. The basic pattern by which the epidemiologic method is turned to practical use in communicable and other disease would seem to hold equally for nutritional disorders. It involves, in order, a definition of the nature and extent of the problem; a search for factors of causation, of the disease and its distributions, through study in nature as populations are affected; formulation of principles for a program of control, these based on demonstrated cause and directed towards death, defect and disability; and finally, field or statistical analysis of results from control measures.

The epidemiologic method is thus seen as an instrument for the study of disease, and also as a medium by which the scientific method is applied to public health. Illustrations from field experience are preferred to didactic discussion in further elaboration of this program for practice. Examples will be cited from each of the three broad fields of interest already stated. As a consequence the present purpose is served by presenting a few principles that bear on the parts of the procedure.

As for the first, the definition of the problem, the chief requisite is a clear statement of what information is sought, what question is to be answered. The limits of normality and the pattern of disease are to be learned. Incidence or prevalence in time and place, by population fractions, and by environmental factors are determined in terms of death, defect or simple disability.

Through comparing observed distributions as they relate to each other and to the known behavior of the disease under study and of other diseases as well, inferences are drawn and evidence sought by further field study or by experiment in support of an hypothesis of cause. Cause is a concern first of origin and secondly of the course the disease takes in its movements through a population. Acceptance of the doctrine of multiple causation requires a search for causative factors in the whole of the ecologic triad by which disease is determined, which is to say in respect to the inciting agent, the host population involved and the environment in which both are placed.

Principles designed to govern a program for control depend first upon statistical or other determination of the relative significance of multiple causes demonstrated. They depend secondly on the availability or possible development of practical measures for prevention or neutralization of those causes. The procedures by which control is sought, and the administrative conduct of the program are conceived as functions of public health practice.

The evaluation of results derived from a program of control is accomplished by comparative and periodic studies; sometimes by statistical analysis of reported data, often by field study because data are not available, and sometimes by both. The nature of the epidemiologic method requires generalizations from a mass of data. The generalizations may be wrong or the program of prevention erected on a false premise, or the prescribed means for control faultily executed. There is need to review accomplishment and appraise results.

The ultimate value derived from a field investigation of disease is believed to depend on an appreciation of the objectives just stated, on the design of the study to accomplish them, and on the technical competence with which the work is done. There is need for proper selection of controls, the choice of an adequate sample, and for appraisal of field methods of measurement including how well a factor may be measured as well as what and what not to measure. Some of the details that bear on these considerations will be sought through review of current and older field activities in nutrition and nutritional diseases.

Field Activities in Nutrition. The purpose now is to outline varieties of field study used in nutrition, to compare methods of approach and techniques with current practice in other mass disease, and to suggest opportunities for a closer relationship between field studies in nutrition and the general problems of public health. No disease lives wholly by itself in this world. In varying degree each impinges on the other and of the dozen or so categories in which preventable conditions may be grouped[32], those of the nutritional disorders and the neuropsychiatric disturbances are judged to have the most pronounced influence on mass disease in general. The remarkable gains in knowledge during recent years have been incentive enough for workers to center effort on the specific problems of nutrition and so far as field studies are concerned "most published results of nutrition surveys indicate that the purpose was to discover deficiences of vitamins"[89]. An extension of field activities to broader epidemiologic interests conceivably would be to the advantage of nutrition and of public health generally.

The term nutrition survey, as used by nutritionists, is a generic expression for all field study, some of which the epidemiologist would recognize as field surveys and some not. All involve collection of data in the field. There are 6 general categories.

Case finding is the simplest of all epidemiologic procedures and much used in nutritional diseases. Recently it has been enlarged to include controlled trial and evaluation of treatment by specific nutrients[91], of persons discovered by the survey to have nutritional deficiency. The pattern of procedure is thus a complete analogy with the usual epidemiologic approach to syphilis. The purpose is to find cases and bring them to treatment. Observations are without limit of time and involve no specific selection of groups for study, nor comparison with control or other groups. Groups of persons judged by clinical or other evidence to be more than usually affected are the usual choice for study.

The field survey in the epidemiologic sense is the examination of selected samples of a population within a prescribed and limited time, with the purpose of determining quantitatively the frequency of existing cases of a disease. This is the short term, cross-section, prevalence study. It commonly involves comparison of observed results with similar situations in other population groups, or with an accepted normal. The usual object is to determine whether the population being studied lies within the limits of accepted normality for the index disease, or exceeds those limits, or lies below. The most common application is in observation of the general nutritional state, in direct appraisal of degrees of inanition but conceivably also in assessment of obesity. It applies as well to specific nutritional deficiency disease. The usual purpose is to learn the requirements for a better program of feeding. It is much used.

A somewhat different procedure also identifies affected members of a population sample, but has as its objective not the determination of the existing total frequency, but estimation of the rate at which new cases appear, within successive periods of time, commonly years. This is the long term incidence study and in combination with prevalence determination serves to determine the dynamic behavior of a disease. Technically, epidemiology recognizes this study of incidence as the field review, in contrast to the short term prevalence study which is the field survey. In nutrition, incidence in terms of cases, or morbidity, has been a rare objective of field procedures, and such information as exists commonly relates to mortality, through use of demographic data.

The techniques of field observation have also been used to good purpose in nutrition for what may be termed administrative purposes, as contrasted to the direct study of disease. Data for groups of persons and for populations have been assembled in respect to food production and food supplies, dietary practices, food consumption and available services for meeting the nutritional needs of populations. They have been used as a means for determining results of public health education and the level of general knowledge about nutrition. From what has been said of multiple causation, studies of this nature contribute also to definition of the causative factors that lie within the social environment, although the direct objective was improvement of the program for control.

Some of the finest accomplishments in the field have been in the investigation of individual nutritional diseases, with the purpose of defining causes of origin, and direction and extent of spread, as mass disease phenomena. Scurvy has been mentioned as a classical example, pellagra will be presented shortly as a pattern of study, and there are many others. This fruitful interest is capable now of much enlargement, as laboratory methods are

increasingly incorporated in field procedure and as the part of the social sciences becomes more clearly demonstrated.

A final interest among field activities is a better understanding of the relation of nutritional disease and the nutritional state to other mass disease. This is seen as distinct from studies which relate these conditions to the general health of communities, as described in the second and third paragraphs above. The problem has 2 parts, first the effect nutritional entities have on the character and course of the particular community disease, and secondly the effect of that disease on the nutritional situation; as specific examples, the part of community nutrition in determining the frequency of diabetes and the role of tuberculosis in determining the nutritional state of populations. These interests actively engage the attention of many from the clinical and experimental viewpoints[83,126]; but the techniques of epidemiology, which appear specially suited to the purpose, are little used.

Techniques of Field Study in Nutrition. Although field methods are now used in the study of many community diseases, in no instance have they been so well summarized or made so readily available as for nutrition. The communicable diseases provide classical illustrations of field method applied to specific problems, and they bring out principle, but no single source comparable to those now to be listed adequately catalogs available procedures and offers critical appraisal of their worth. The 1939 publication of Bigwood from the League of Nations[12] presents method and principle comprehensively and from a world point of view. A number of others may be consulted to advantage[96,105]. A bulletin of the National Research Council[89] of recent months presents American practice critically and in detail. Successive sections are devoted to objectives of nutritional surveys, dietary surveys, chemical tests and their interpretation, clinical diagnosis in nutrition surveys, and the part that surveys have in community nutrition programs, in the training of nutritionists and in the work of health departments. So adequately does it fulfil its purpose in presenting techniques and the value of nutritional surveys, that discussion here is limited to a few epidemiological considerations. This monograph should be consulted for detail and for further guidance through its extensive and up to date bibliography.

Epidemiologic study of disease in the field makes use of 3 principal scientific disciplines, those of clinical medicine, of the laboratory and of biostatistics. This holds equally as the method is applied to nutritional diseases. An understanding of human nutritional requirements was first approached through studies of the diets of persons in apparent good health[121], the dietary survey. As interests were extended to the characteristics of diets that resulted in disease, clinical methods[39] were required to identify the parts of populations affected and to separate those with similar conditions of other origin. As biochemical knowledge of nutrients became available, it was promptly turned to field investigations, either as independent projects in evaluation of diets and their effect on the human host, or more often as useful additions to the existing field methods.

Although 3 lines of development are recognized in field studies in nutrition, the present tendency, as in the communicable diseases, is to conceive field investigation as a team effort, making use of a variety of scientific disciplines and combining all 3 approaches. This

is largely because no one has satisfied all requirements[22].

The medical nutritional survey is limited by absence of any single pathognomonic physical sign of specific nutritional deficiency[89]. The interpretation of clinical findings in an individual patient requires careful differential diagnosis[1], commonly through aid of laboratory tests. Judged in respect to the group, the medical survey has little value unless related to the total environment of the population under study[71a].

As a field procedure applied to appreciable numbers of persons, the dietary survey has the virtue of simplicity, but at best provides a rough index of food intake and of nutrients provided[92b,128]. There is no agreement on the number of days of observation required for reliable results; and such factors as seasonal variations and changing income introduce important variables.

Biochemical evaluation of nutritional status includes in some instances tests which indicate previous dietary intake of nutrients, methods for estimating saturation or depletion of bodily stores of certain chemical substances, and there are tests which measure the metabolically active forms of dietary essentials and the abnormalities which occur in deficiency disease[89]. The practical difficulty lies in the common lack of correlation between biochemical and clinical measurements. This has been the experience in some of the most carefully conducted studies of recent years[104]. Some of the reasons for the observed irregularities have been discussed by Sinclair[120], Pett *et al.*[99] and Darby[23], and most recently by Putnam *et al.*[102]. It is not surprising under the circumstances, that emphasis has been placed on the combined type of nutritional survey in an endeavor to acquire greater precision. Excellent examples within recent times are those of Youmans and his associates[137,138a,b], and contributions by the group working with Milam[84,85].

The nutritional survey has had wide application to a variety of population groups. Some observations relate to normal general populations[84], but more often to school children[85]. Selected industries have been studied[13]; inmates of institutions[77]; troops in the field[62] and such racial groups as African Negro children[65]. Other study groups have been drawn from hospital surgical patients[139], patients with diabetes[68], and normal pregnant women[16,17,18]. Since the purpose of a nutritional survey commonly requires selected populations, it is clearly necessary to state the basis for the selection and to define the group studied. The nature of many populations makes avoidance of a biased sample difficult, but the frequency of this fault in field nutritional studies suggests also a lack of familiarity with the principles of sampling now available in a number of manuals[51]. Muench[88] and Putnam *et al.*[102] have given attention to the matter as it relates to nutrition, and the British studies on hemoglobin[81] are especially informative of measures to be taken to assure adequacy of sampling.

Nutritional Status of Populations. The three principal interests of field activities in nutrition have been stated as the assessment of the nutritional status of populations, the study of specific nutritional diseases, and measurement of the influence of nutrition on other mass disease. The first is important because it provides the definition of a normal upon which depends the direction of effort in the other two fields. It aims further at an interpretation of inanition and malnutrition. The problem would seem simple enough; first to determine a normal, and whatever lies below that is malnutrition.

The principal difficulty lies in assessing a normal[110]. In the first place, there is every reason to believe that the healthy individual can adjust to a wide range in dietary intake. Beyond that, the lesser signs of deficiency disease may be expected to appear. By reason of evidence already presented, the determination of the range of normality is further complicated by lack of good correlation between clinical and biochemical observations. Any reliable concept of malnutrition is intimately associated with the methods and criteria of evaluation. The result has been a variety of standards based on food intake, on disturbances of metabolism and on height-weight relationships. Various attempts have been made to bring forth acceptable standards[115]. If the answer is to be found in terms of nutritional characteristics, that is a continuing obligation of students of nutrition. The added possibility exists that the answer is in another kind of measurement, wholly outside nutritional standards.

A second difficulty in evaluating the nutritional status of populations rests in the unsatisfactory definition of a biologic gradient for most nutritional conditions. As with disease in general, it is the lesser degrees of disability that give most trouble, the slight deviations from normal which also are more frequent than full-blown manifestations[24]. In practice, differences in degree are often distinguished by such unsatisfactory comparative terms as good, unsatisfactory and bad, and all undefined. Some gain in differentiation is made by using "No abnormality detected", "Requiring medical and nursing care", or "Requiring hospital care". There is general need for a clear description of pathologic differences, comparable to those used in diphtheria.

A review of nutritional surveys suggests the benefit to be had from principles well devoloped in the study of mass disease generally. The epidemiologist commonly places much weight on time-place-person relationships, with the result that he is commonly distressed by the generalizations made in nutritional surveys on the basis of single examinations. That conditions of food intake and availability of food supplies vary with season is not unappreciated in nutrition[128] and material differences have been demonstrated in the nutritional state of identical populations as determined in autumn and spring[66]. Place would appear an equally important consideration, for Johnson and Kark[57] have demonstrated nutritional requirements in tropical areas to differ materially from those of temperate zones and those of the Arctic are of still another order[63]. The principle that children during the period of growth are most susceptible to nutritional inadequacies and that similar effects are shown during pregnancy and lactation is sufficient indication that the nutritional requirements of a population are significantly affected by differences in age composition. Systematic study of selected nutritive disorders in the same population under varying environmental conditions, where environment is broadly interpreted in its physical, biologic and social components might well contribute to better understanding of the normal nutritional state.

More universal use of some of the technical refinements of the epidemiologic method could also contribute to results from nutritional surveys. Rates of prevalence and of incidence are commonly used together in determining the dynamics of mass disease of populations. This procedure is particularly useful in diseases of slow development and long persistence, the two values often being widely divergent. An altered frequency of attack commonly brings a change in this relationship. Incidence

is expectedly high in an evolving process, early in epidemics, sometimes closely approximating prevalence. In a period of decline, incidence may be low or almost negligible, and yet prevalence is at a level far in excess of normal expectancy. Nutritional field studies commonly compare rates of prevalence determined at intervals, but the combined indices are not so frequently used as in the communicable diseases[100].

Increasing reference is found in the literature of nutritional surveys to the need of an analyst. The greater need would appear to be a "synthesist". Too many surveys are organized on the premise of collecting all possible data pertaining to the situation, and then calling in a statistician to find out what has been discovered. The basic need is a proper design of experiment before operations begin, first to state clearly the question to be answered and second to develop a planned procedure to accomplish that objective. The required skill involves more than mathematics, and assuredly more than retrospective figuring.

A third fundamental in any survey of appreciable extent is a pilot investigation designed to test the efficiency of methods and the inclusiveness of the kinds of data sought.

Some of the difficulties in determining a normal state of nutrition conceivably could be solved by demonstration of a means of measurement that lies outside strictly nutritional characteristics, an objective test of function in relation to food intake. This would be a test of what a community could do on a particular dietary regimen, rather than what the regimen is or the mark it leaves on a population as signs of disease. Indices that might be explored are resistance to selected infections, based on demographic data[26], on economic productiveness where exceptional correlation has been demonstrated in at least one instance[69], or on general morbidity and mortality. The newer techniques of the social sciences might provide a quantitative evaluation of the decreased efficiency of populations which was suggested so strongly as an aftermath of nutritional difficulties in Holland and Belgium at the close of the recent war[26]. A particular study of the moment is directed towards appraising obesity among employees of a large industry, where overweight is being correlated with absenteeism, the prevalence of accidents and administrative efficiency ratings.

Epidemiology of Pellagra. The epidemiologic approach to an individual disease, which has been set forth as the vertical direction of effort, provides a choice of 4 general procedures. The oldest is based on medical geography, with an examination of the behavior of a disease in time and place by the methods of historical and geographical pathology[52]. Under other circumstances the start is in the experimental laboratory with the results of biochemical or other investigations transferred to the field for practical test in relation to populations of people[58]. The statistical approach makes use of demographic data on reported cases and deaths, and, again using the fundamental concept of time, place and person relationships, seeks to determine the relative weight of apparent causes[2]. Finally there is the field method of study[1,5], the most direct and individual of epidemiology, by which the causes of disease in population units are sought through observations in nature according to a designed plan. Because pellagra so well fits the present purpose of illustrating method, it is selected as the disease to be considered and the historical-geographic approach is emphasized, although all 4 procedures are seen to have entered into the developing fabric of knowl-

edge, as they usually do, and any one of a number of nutritional conditions might have been chosen to equal advantage.

Knowledge of Pellagra in 1900. Pellagra was first reported[45] in the United States in 1864. The first serious outbreak[118] was in 1905. By the time pellagra became a conspicuous public health problem in America[25] early in the twentieth century, considerable information had already accumulated, this despite the fact that it clearly was no old and long established disease in the sense of leprosy, mumps, typhoid fever and many others. Casal had first observed pellagra in Spain about 1730 and described[19] it as a disease of relatively recent evolution. World experience substantiated that opinion, for even in regions that became heavily affected as in Italy[48], there was no previous record of its occurrence. The inference reasonably followed that it was due to some recently introduced factor.

The physical environment was seemingly concerned because the disease was restricted to latitudes of 42° to 46° N. and its epidemic manifestations were those of a spring time disease. The irregular occurrence of pellagra within this zone pointed to the probability that it developed from causes that acted locally; and that these could not be matters of climate, weather or the soil, for they had always been present while the disease had not. Furthermore, similar conditions characterized regions where pellagra was not seen.

The influence of the social component of the environment was marked by the common association with poverty, and the greater frequency in rural than urban communities. While pellagra was regularly connected with the poorest parts of a population, actual conditions were often no worse than in regions that were without pellagra. It was never possible to relate notable social changes with the introduction of the disease. In Italy the better parts of the country, Lombardi and Venezia, were most heavily affected. Poverty alone evidently was not the deciding issue.

A host factor was manifest in the disproportionate attack rates between men and women and the extent to which incidence centered in the older age groups.

There was firm opinion and a goodly amount of evidence about the direct agent. It had something to do with poor food and was generally believed related to maize. One important observation was that pellagra had not been known in Europe before the introduction of maize in Italy about the middle of the 16th century. Pellagra was no community health concern unless corn was an important part of the common dietary. In France a change in agriculture[108] by which the cultivation of other grains was substituted for maize had led to disappearance of pellagra. The case for maize as the agent of the disease was good, and yet some of the evidence was against that hypothesis. The maize zone of the world was wide and the distributions of pellagra circumscribed. The disease did not appear until many years after maize was introduced, commonly two or more generations, but this could be explained by the time required for its use to become general and appreciable. The sporadic cases that occurred where maize was not a general food were difficult to explain; and recourse was had, either to interpret them as errors in diagnosis or to recognize another disease entity, with the result that eventually an extensive literature developed about pseudo-pellagra. Doubt persisted in the minds of some that maize was the whole answer. Other grains or other dietary factors might be involved, or the cause might even be non-dietary, but in general the search was for the

specific agent, or the mechanism by which corn caused pellagra.

The Agent of Disease. Acting on the hypothesis that pellagra was due to an exclusive or preponderant diet of maize, limited nutritive values was clearly not the answer. Pellagra does not follow from too little food, although other symptoms may. Pellagrins often look well fed. Other populations subsist on a diet principally of rice or potato that is no better in terms of calories, and yet they do not suffer from pellagra. Presumably there was something special about corn. A remarkable feature of the hypotheses brought forth in explanation of the cause of pellagra during the two centuries that the disease has been known, is the extent to which they reflect the prevailing thought of the day.

The late nineteenth century was an era when the deleterious action of food commonly was attributed to poisonous action. This was the day of the ptomaines and ergotism. It is, therefore, not unexpected that the prevailing explanation of pellagra was that toxic substances developed in maize through decomposition, that damaged maize caused disease, and that corn fully ripened and sun dried was without effect. Pellagra was most common in countries where conditions were least suited to corn cultivation. A bad maize crop was often associated with a bad year of pellagra. Corn cut before ripening or stored without drying or under damp conditions was susceptible to mould. Balardini[7] isolated a green mould from spoiled corn and with it produced acute symptoms of disease in man and animals. Pellagra, however, is a chronic rather than an acute disease, and the same mould was demonstrated in regions where pellagra did not exist and it was not commonly present in many pellagrous districts. Other toxic factors were periodically advanced as the agent of disease in pellagra, but proof was invariably poor and interest was lost in a new enthusiasm about infectious agents as a likely cause, in the period during which bacteriology saw such great development.

Scattered reports of nondescript microorganisms resulted in no serious attention to a possible infectious agent in pellagra until the reports of the Illinois Pellagra Commission[103] and of the Thompson-McFadden Pellagra Commission[119] supported that mode of origin. In neither instance was a specific pathogenic microorganism identified. The investigations of the second group combined field studies in South Carolina with experimental observations in base laboratories. They were beset with difficulties, in that patients brought north to provide a satisfactory source of materials promptly improved on the good hospital diet; and monkeys injected with suspected intestinal bacteria developed disquieting reactions. These investigations and the active interest in arthropod transmission of infectious agents then current doubtless influenced many attempts of the period to demonstrate arthropod vectors of pellagra. The spotty distribution of the disease and the assured fact that it is not transmitted from person to person favored such an hypothesis. Mosquitoes[106], flies[56], and other insects were variously incriminated but none with such ease as the buffalo gnat whose part was defined by Sambon[113] through field studies that involved no more than a stop between trains in Italy. Meanwhile Hopkins[53] had made his classical contributions to the knowledge of "accessory food factors", and interest turned to the field of nutrition. Goldberger[42] undertook his famous field studies in the mill villages and prisons of the South and, having demonstrated that pellagra could be cured by diet, he reasoned that cause must be within diet. That he proved, in a series of

sound laboratory and field investigations. Pellagra became established as a dietary deficiency, thus marking the third phase through which investigations passed.

The experimental demonstration of a pellagra preventive factor[41] as a part of what is now known as the vitamin B complex was the initial gain in the fourth and current phase of knowledge about pellagra. Elvehjem's[31] classical contribution demonstrating nicotinic acid deficiency as the direct causative factor in canine black tongue was shortly followed by a series of reports[37,109,123,130] that confirmed its similar place in the human disease pellagra. Subsequent work showed that dietary deficiency in niacin (nicotinic acid) could be compensated in a measure by another dietary component, in that tryptophane functions as a metabolic precursor of niacin in the rat[70,179] and in man[116]. Corn lacks adequate amounts of both the vitamin niacin and its precursor tryptophane. There is also the possibility brought out in the recent suggestion that corn acts also through an antivitamin[136] interfering with the normal metabolic activities of niacin.

The Host Factor. Some features of the complex that constitutes the host factor in causation of pellagra are well known and supported by good evidence[49]. Others are suggested by clinical experience and yet have not been subjected to systematic investigation by quantitative methods. The inherent and fundamental characteristics that rest in age, sex and race are among the better known.

The factor of age as it applies in most deficiency diseases is expressed in a principle that susceptibility is greatest during periods of growth, during reproduction and in the course of lactation[71b]. Some experimental deficiences are only produced under such circumstances, and regularly that is the favored approach. Pellagra provides a paradox in that older persons are principally affected. Age relationships in mass disease are determined either by mortality or morbidity, mortality usually serving because of the greater availability and exactness of the information. Each has its use. The mortality for beriberi in the Philippines[112] in 1947 was 132 per 100,000 population; it is the second largest cause of death, tuberculosis being the first. Two thirds of all deaths were of infants, while field surveys showed the greatest prevalence of cases in population groups representing in order, expectant mothers, nursing mothers, other adults, infants and finally children. Mortality of pellagra in the United States is heavily weighted at older ages (Fig. 1), but morbidity is also a function of older people[25]. The older age of attack in pellagra has never been satisfactorily explained. Pellagra is recognized as a chronic disease, but why in contradistinction to other common vitamin deficiencies? It could be due to children being protected by milk diet, but the same age distribution holds for regions where milk after nursing is not a prominent feature of the diet. The discrepancy may rest in failure to define carefully the gradient of disease, with an unrecognized form of pellagra being frequent among infants. The recent work of the Gillmans[40] suggests that possibility.

Attack rates in families are invariably greater for females than for males[25] which is reasonably related to differences in food intake because of occupation, habit and similar factors. That observed racial differences are dependent on other than genetic considerations and that the social environment is the principal factor is supported by American data where the ratio of occurrence between negro and white has progressively approached parity (Table

1) after an original wide discrepancy and as environmental conditions tended to equalize.

The common interpretation of acquired host factors in respect to ability to resist disease is that expressed by specific immunity to infection. The anatomic and physiologic changes that arise from a wide variety of diseases are often an equally important consideration among causes that operate to produce other morbid processes, not only infections but many other diseases. Pellagra is a good illustration. Alcoholism has a well-recognized influence[124], as have gastrointestinal diseases[11]. Sporadic cases are commonly heavily weighted by host factors identified with inability to assimilate and utilize a normal diet, as distinguished from the grouped occurrence of cases which is characteristic of a common inadequacy of diet.

Individual idiosyncracy, mental depression and other factors of the psychic constitution are commonly seen as causative factors in pellagra and in other deficiency diseases.

The Environment. The factor of environment in all its components enters

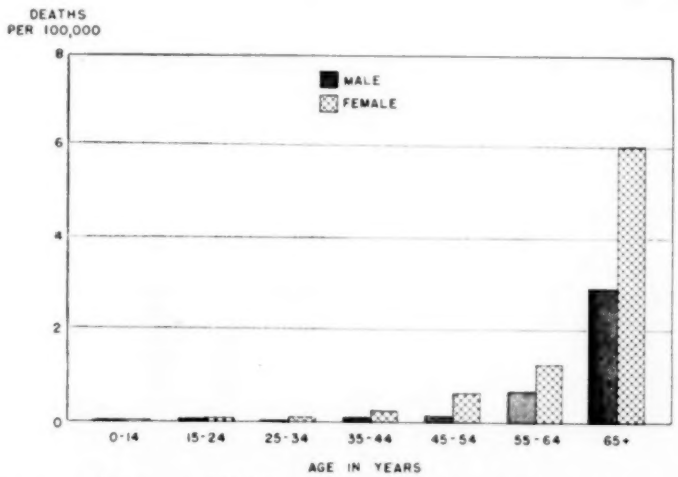

FIG. 1.—Pellagra, mortality per 100,000 population, by age and sex, United States, 1947. Source: Data of Vital Statistics—Sp. Reps. **31**, No. 11, 1949 (National Office of Vital Statistics, U. S. Pub. Health Serv., F. S. A.).

TABLE 1.—PELLAGRA, MORTALITY PER 100,000 POPULATION, BY RACE, FIVE SOUTHERN STATES, U. S. A.

(Alabama, Georgia, Louisiana, Mississippi, and South Carolina)
1930–1947

	Population		*Mortality*		
Year	*% Negro*	*% White*	*Negro*	*White*	*Ratio Negro/White*
1930	40.3	59.7	45.1	13.1	3.4
1935	40.3	59.7	15.8	7.4	2.1
1940	42.2	57.8	10.6	5.9	1.8
1945	42.2	57.8	2.9	2.5	1.2
1947	42.2	57.8	2.3	2.0	1.2

Source: Data of Vital Statistics of the United States (National Office of Vital Statistics, U. S. Pub. Health Serv., F. S. A.)

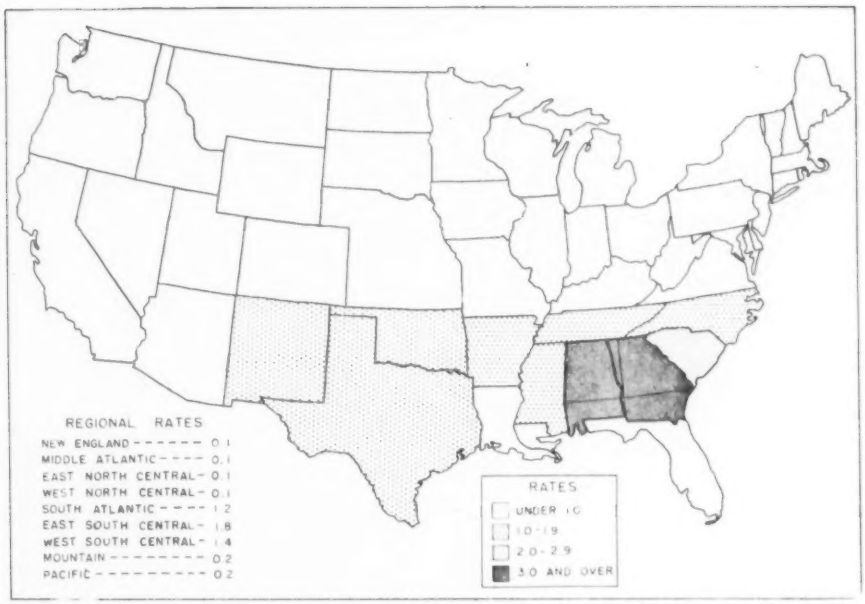
REGIONAL RATES
NEW ENGLAND - - - - - - 0.1
MIDDLE ATLANTIC - - - - 0.1
EAST NORTH CENTRAL - 0.1
WEST NORTH CENTRAL - 0.1
SOUTH ATLANTIC - - - - 1.2
EAST SOUTH CENTRAL - 1.8
WEST SOUTH CENTRAL - 1.4
MOUNTAIN - - - - - - - - 0.2
PACIFIC - - - - - - - - - 0.2
RATES
UNDER 1.0
1.0-1.9
2.0-2.9
3.0 AND OVER

FIG. 2.—Pellagra, mortality per 100,000 population, United States, 1947. Source: Vital Statistics of the United States—Sp. Reps. **31**, No. 4, 1949 (National Office of Vital Statistics, U. S. Pub. Health Serv., F. S. A.).

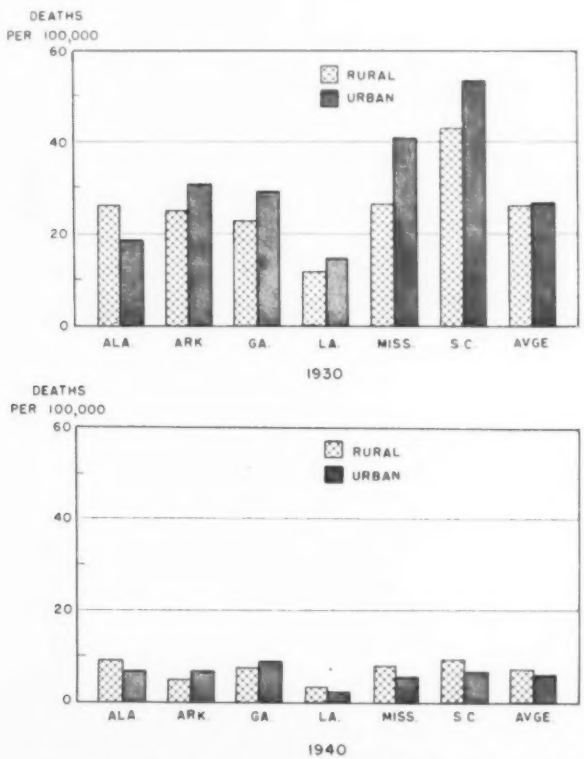

FIG. 3.—Pellagra, mortality per 100,000 population, rural and urban, in six southern states, 1930 and 1940. Source: R. E. Remington: The Enigma of Pellagra, So. Med. J., **37**, 605, 1944.

into causation of pellagra. The world center of prevalence is well defined geographically. In the United States pellagra is essentially a disease of the southern states (Fig. 2). The time factor is demonstrated both in a seasonal incidence which characterizes April and May and in larger secular variations which in the years pellagra has been recognized in this country have shown two principal epidemic waves, one during the years 1910-1915 and the other in the late part of the 1920 decade. Mortality has decreased materially in recent years (Fig. 3).

The biologic component of environment has been repeatedly demonstrated in the history of pellagra, through common correlation of poor corn years and a heightened frequency of the disease during the subsequent winter.

Appreciation of the social environment as a factor in the frequency of pellagra dates from earliest knowledge of the disease[6]. It has had repeated confirmation in the association of pellagra with poverty[125]. Originally a rural disease, it became more common in cities with the movements of population to an urban existence, but in recent years (Fig. 3) has returned to its original rural prominence. The extent to which varying features of host and environment enter into causation of nutritional and other diseases finds no better illustration than the manner in which pieces of evidence were put together in determining the cause of pellagra. The peculiar old lady who lived in the manor house, the family down by the tracks and the warden of the penitentiary all reported similar difficulties that pointed the way to a common cause, dietary deficiency, and yet they arose under a variety of environmental circumstances.

Nutrition and Other Community Disease. Nutritionists have long recognized that malnutrition was caused by a number of factors other than inadequate dietary intake[34], that it may occur in the presence of otherwise adequate dietaries by reason of interference with the normal processes of absorption and utilization, or through increased nutritional requirements or destruction or excretion of nutrients. A literature has developed on "conditioning factors"[59] which have been the subject of recent reviews[101,127]. Gastrointestinal disturbances are commonly concerned[76,134], together with numerous infections and parasitic diseases. To those who look upon the pathogenesis of disease and the genesis of mass disease in communities from a holistic point of view—and epidemiologists surely must—it is at least unusual that the situation is so regularly viewed in terms of the nutritional disturbance and not of the other diseases involved; this despite frequent evidence that the nutritional disorder is neither the primary condition nor the more important. An approach through study of the effect of nutrition on a variety of mass diseases of man, in contrast to the effect of these diseases on nutrition, is logical and conceivably of much public health benefit. Clinical studies of this nature have had to do with surgical infections[139] and with nutritional disturbances among patients with diabetes[68]. The value of exploring the larger problem that relates to populations is suggested in a probable relationship between community overeating and the frequency of community diabetes; and yet few observations of this kind have been made for any disease[28].

Until recently, lack of satisfactory method has made impractical studies on the effect of diet on general resistance to infection, or of malnutrition as a feature of susceptibility. Most work has been with the techniques of experimental epidemiology[117a,117b]. Improved epidemiologic method and the signifi

cant addition of biochemical techniques for determining nutritional levels make field studies practicable in obtaining further evidence on the hypothesis of Aycock and Lutman[4] that vitamin deficiency is not an epidemiologic principle in infections.

The opportunities are by no means limited to such mass disease as infections and metabolic disturbances which are rather clearly related to nutrition. Others such as cancer and arteriosclerosis are known to have a significant host factor, and part may well be resident in nutrition. The demonstration of such relationships would justify more than anything else a wider use of the nutritional survey and its incorporation into general public health practice. A different sort of nutritional survey than that now commonly used would be essential to these purposes, where field techniques would combine present methods with examinations for other mass diseases of populations; in essence, a health survey built around nutrition. Actual development of such methods is under way not only in America but in such diverse places as the Fiji Islands[9], in South Africa[64] and in the Netherland East Indies[27]. Under such circumstances, the common statement of objectives would be altered from "the role of surveys in community nutrition programs" to read "the role of nutrition surveys in a community health program."

Summary. Field studies in nutrition initially were concerned almost solely with food consumption and its relation to the health of populations. The method was the dietary survey.

The identification of specific nutrients and the better definition of nutritional disease entities led to a second phase in field activities, where the chief concern was with the prevalence of individual dietary deficiencies and of specific nutritional disorders. Laboratory tests were incorporated into field practice, and clinical methods attained a new level of importance and an added perfection.

A third phase in the direction of field interests is the current tendency to broaden the nutritional survey and to use it as the foundation for a general health survey. The concern is less with nutritional disorders and the nutritional state as such, and more with the part they have in determining the character and behavior of other mass disease, which is increasingly appreciated as material. Nutrition thus follows the course of most other fields: an initial emphasis on its own individual problems and an eventual incorporation of those interests into the general pattern of medical and epidemiologic effort toward health and welfare of populations. In the case of nutrition, it is actually a return to the original objective of the dietary survey, but with much better methods and a greater promise of accomplishment.

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PROCEEDINGS OF

The Physiological Society of Philadelphia

SESSION OF JANUARY 17, 1950

Vasopressor Components of Pheochromocytomas. KARL H. BEYER, Ph.D., M.D., CHARLES A. ROSS, WILLIAM S. WALLER, and VIRGIL D. WIEBELHAUS, Ph.D. (Dept. of Pharmacology, Med. Res. Div., Sharp and Dohme, Inc., Glenolden, Pa.). Two pheochromocytomas have been assayed chemically for their epinephrine-like content by the method of Beyer and Shapiro (Am. J. Physiol., **144**, 321, 1945). Tumor A contained 6.9 mg. of a β-catecholethylamine, calculated as epinephrine, per gm. of tissue. This tumor was submitted to us by Dr. R. L. Mayock of the University of Pennsylvania Medical School. Tumor B contained 8.17 mg./gm. of tissue. This gland was submitted by Dr. W. S. Randall of the Ochsner Clinic, New Orleans. Hydrolysis of the extracts did not increase the amine concentration. The amines were ultrafilterable from the extracts.

Filtered extracts of the tumors were compared with mixtures of l-epinephrine and l-*nor*epinephrine for pressor activity in the dog before and following the administration of dibenamine. Tumor A contained 10.3 mg./gm. of pressor material and tumor B contained 11.8 mg./gm. of tissue, calculated as epinephrine. Whereas the pressor principle of tumor A was about 50% *nor*epinephrine, the pressor component of tumor B appeared to be as much as 90% *nor*epinephrine.

Incubation of tumor extracts with catechol oxidase or with a crude amine oxidase preparation destroyed all pressor activity of the extracts.

These experiments indicate that the immediately available pressor material in these tumors exists in a free, active and readily diffusible state. The unusually high *nor*epinephrine content of the tumors is consistent with the view that it is the immediate precursor of epinephrine and that transmethylation of this precursor is impaired in the tumor, as has been suggested by the reports of others. The pressor activity of these tumors could be accounted for by the presence of β-catecholethylamines, and possibly their precursors. The pressor action could be reproduced by mixtures of l-epinephrine and l-*nor*epinephrine.

The Action of Hyaluronidase and Steroids on Membrane Permeability. JOSEPH SEIFTER, M.D., DONALD R. FITCH, M.D., DAVID H. BAEDER, A.B., and ALBERT J. BEGANY, A. B. (Wyeth Institute of Applied Biochemistry, and Hahnemann Med. College and Hospital, Phila.). It has been demonstrated that the permeability of lens, urinary bladder, and synovial membranes is increased by hyaluronidase and by desoxycorticosterone, and decreased by the alarm reaction, adrenocorticotrophic hormone, and certain physiological steroids, the most effective of which is cortisone. Cortisone counteracted the increased permeability effects of either hyaluronidase or DOCA. Local application of hyaluronidase to the synovial membrane or the systemic administration of DOCA simulated the initial lesion of arthritis. This condition was alleviated by steroids which are anti-DOCA and anti-hyaluronidase.

One of the therapeutic effects of cortisone in arthritis was considered to be an immediate anti-enzyme or anti-DOCA effect on the synovial membrane (end organ) resulting in alleviation of edema and swelling. The rabbit synovial membrane technique was employed to screen steroids for cortisone effect on the end organ of arthritis, and to test their anti-hyaluronidase and anti-DOCA properties. The activity of a standard dose of cortisone acetate was taken as 100%. The normal membrane in balance with DOCA and cortisone type steroids had a cortisone ratio of 5:2. Of more than 50 steroids screened, 38 were found to have anti-permeability action less than cortisone. Eleven analogues of DOCA were demonstrated to be at least as active as cortisone and to have anti-DOCA and anti-hyaluronidase properties. Artison (Δ 5, pregnene, 3β, 21 diol — 20 one — 21 monoacetate, Wyeth) has been placed on clinical trial and was successful in 80% of arthritics treated and in one case of scleroderma. Six compounds which are not structural analogues of DOCA had a high cortisone ratio. Two of these have been placed on clinical trial and found effective against rheumatoid arthritis. Steroids appear to act on ground substance by controlling the degree of polymerization of the ground substance. Practically all steroids had some degree of anti-permeability action on the synovial membrane. No steroid had the permeability effect of DOCA.

Location of Receptors for Tonic Neck Reflexes. G. P. McCouch, M.D., T. H. Ling, M.D., and I. D. Deering, M.S. (Dept. of Physiology, Univ. of Penna.). In 6 decerebrate cats, showing tonic neck reflexes to turning the head, both internal ears were destroyed and all muscles connecting head with neck or atlas with axis were severed. In 2 of these the spinal accessory and the first 3 cervical nerves were cut at their entrance into muscle. In all 6 animals the reflex to turning the head was retained after these procedures.

In 6 decerebrate cats, showing tonic neck reflexes to turning the head, both internal ears were destroyed and the first 2 or 3 cervical nerves were exposed with as little dissection of muscle as possible and with complete retention of innervation. Tonic neck reflexes were not impaired. An attempt was then made to denervate the upper neck joints by circumsection of the first 2 or 3 cervical nerves at their emergence through the intervertebral ligaments without injuring the muscular branches. When this was done unilaterally, the neck reflex to turning the head was abolished in the ipsilateral extremities; when bilaterally, it was absent in all 4 limbs.

It is concluded that receptors for the tonic neck reflex to turning the head are located in the joints of the upper neck, especially the atlanto-axial joint. If there be muscular receptors which facilitate this reflex, their effect in isolation is subliminal under the conditions of these experiments.

Observations on the Reflex Regulation of the Circulation. Lysle H. Peterson, B.S. (Dept. of Physiology, Univ. of Penna.). A number of surprising responses of the circulation following stimulation of various reflex systems have been observed in over 600 normal subjects and hospital patients during physiological experiments and surgery. Certain reflex responses appear to differ in man as compared with other species, to differ also with and without anesthesia. Following carotid sinus stimulation there appears to be a primary weakening of ventricular contraction concurrent with bradycardia. The effects are in contrast to those follow-

ing prolonged diastole following premature or missed contractions. Following stimulation of the mesenteric receptors there also appears to be a primary weakening of contraction independent of pulse rate. Evidence is also presented to show that under various physiological, pathological and anesthetic conditions there is a marked alteration in the manner in which the cardiovascular system reacts to changes within itself. These data are presented not only to demonstrate reflex circulatory phenomena in man (some for the first time) but also to stress the need for re-evaluation of present concepts of circulatory reflexes. Such generalizations which are commonly stated as Starling's and Marey's laws and the belief that the ventricles are not directly affected by the vagi must be thought of with reservations. An understanding of reflex balance in man seems necessary for an understanding of diseases of the cardiovascular system of man.

BOOK REVIEWS AND NOTICES

MODERN PRACTICE IN OPHTHALMOLOGY. Edited by H. B. STALLARD, M.D., St. Bartholomew's Hospital, London. Pp. 525; 231 ills. New York: Paul B. Hoeber, 1949. Price, $12.50.

THIS is a text for the general practitioner and for medical students written by 14 well known British ophthalmologists. The first chapters deal with the anatomy, physiology and pathology of the eye. Chapters on methods of examination and ocular therapeutics follow; the rest of the book presents the diseases according to the part of the eye chiefly affected.

Like many books by many different authors, this one suffers from a lack of uniformity. The chapter on medical ophthalmology is the largest, as it should be, but is hardly adequate for the general purpose of the book.

F. A.

AN ATLAS OF THE BLOOD AND BONE MARROW. By A. PHILLIP CUSTER, M.D., Ass't Prof. of Pathology, Univ. of Pennsylvania. Pp. 321; 285 ills., 42 in color. Phila.: W. B. Saunders, 1949. Price, $15.00.

LONG before this book appeared I knew that it would be a good one and one, too, that must be on one's shelves for ready consultation. How, then, to review it objectively—the work of a co-worker and close friend for over 20 years? Obviously, at least it should be scrutinized with the greatest care, glossing over no imperfections and giving praise where indicated in carefully measured terms. To put this into effect, however, when faced with the familiar terms, principles and concepts, and even much of the material that had grown up during these years, is a task that proved unexpectedly difficult. Nothing of significance is found to criticize unfavorably and much to praise. In an attempt to gild the lily, however, the suggestion is offered that all legends, instead of a very few, could advantageously have included the kind of stain used, suitably abbreviated of course. Even the price, in view of the number of excellent large black and white and colored illustrations, is not unreasonable.

As is proper for an Atlas, the clearly written text appears as an accompaniment of the illustrations, fulfilling the book's prime function to promote accuracy in diagnosis. Also quite properly the nature of the diseases presented is not considered, though significant clinical features and supporting laboratory tests are included to broaden the basis for diagnosis.

The brief Part 1—The Hemolytopoietic System—includes in its 34 pages 3 chapters on hematopoiesis, and the constituents of normal blood and bone marrow. The rest of the book treats the various blood disorders, classified into 12 groups.

"Clinicians, clinical hematologists, pathologists, and medical technologists", for whom the book was planned, should indeed find it useful; if they don't, they had better shift to another field.

Both Author and publisher should indeed be proud of this addition to a rapidly growing field.

E. K.

HUMAN PATHOLOGY. By HOWARD T. KARSNER, M.D., Former Prof. of Pathology, Western Reserve Univ. 7th ed. Pp. 927; 562 ills., 24 color plates. Phila.: J. B. Lippincott, 1949. Price, $12.00.

APPEARING after an interval of 7 years—the longest between any of the previous editions—this one has had more extensive revision than any of its predecessors. It also has 241 new illustrations, about equally divided between additions and replacements. These are all good; very few of the diagrammatic 19th century variety that marred the earlier editions now remain. The 800 new references (requiring the deletion of 900 older ones because of space requirements) reinforce the reference value of the book, which has always been one of its stronger features. With continuation of the already established trend toward inclusion of more pathologic physiology, "Karsner" entrenches itself even more firmly as one of the world's two or three leading textbooks of pathology.

E. K.

MODERN PRACTICE IN ANAESTHESIA. Edited by FRANKIS T. EVANS, M.B., Honorary Anaesthetist, St. Bartholomew's Hosp., London. Pp. 606; 227 ills. New York: Paul B. Hoeber, 1949. Price, $12.50.

ANESTHESIOLOGY today has many facets. No single individual can encompass the field. This new book, with a single editor but with multiple contributors, should therefore be well designed to assess "modern

practice in anesthesia". Unfortunately, in the Reviewer's opinion, the appraisal is inadequate. There are errors; it is wrong to say that atropine in doses of 1/200 grain should be given to infants under the age of 6 months, or to state that neurological sequelae to spinal anesthesia are more theoretical than real. Recent advances in physiology and pharmacology are not included, the authors apparently relying almost entirely on clinical journals as source material. To be commended is the inclusion of such topics as posture in anesthesia, blood transfusion and replacement therapy, anesthesia and heart disease (written by a cardiologist). The format is attractive and for those desiring a source of British material the volume may prove useful. R. D.

CARDIOVASCULAR DISEASE. By LOUIS H. SIGLER, M.D., Chief of Cardiac Clinic, Coney Island Hospital. Pp. 551; 149 ills. New York: Grune & Stratton, 1949. Price, $10.00.

FROM the opening chapters on incidence and etiology to the closing chapters relating to pregnancy and surgery, this very readable book adequately considers the important aspects of cardiovascular disease. The Author stresses physiologic phenomena, presenting much that is difficult and controversial in a clear, simplified, and impartial manner. For those interested in more detailed study, each chapter has a useful bibliography. The Author avoids special consideration of electrocardiography by referring to his own book on the subject.

In a field where several good texts are available, this new book earns its place by providing a fresh presentation that is at once fairly complete, but not burdensome to the reader. To this Reviewer, the work is especially worth while for the Author's correct description of the murmur of mitral stenosis and his laudable omission of the customary but inaccurate adjective, "mid-diastolic." H. Z.

STARLING'S PRINCIPLES OF HUMAN PHYSIOLOGY. By C. LOVATT EVANS, D.Sc., Jodrell Prof. of Physiology, University College, London. Chapters on *The Special Senses* by H. HARTRIDGE, M.D., Inst. of Ophthalmology, London. 10th ed. Pp. 1193; 693 ills., some in color. Phila.: Lea & Febiger, 1949. Price, $10.00.

THIS volume is the best extensive one man product of its kind that the Reviewer has met. Except for the special senses, the entire book comes from the pen of Professor Evans. It exemplifies the virtues and the limitations of such an undertaking. On the credit side: a well integrated treatment free from overlapping and unnecessary repetition and a uniformly clear and distinguished style that make reading a pleasure. Especially commendable are the historical introductions to the separate sections of the book. On the debit side: uneven validity of various parts in critical viewpoint, in selection of material and points of emphasis, and in degree of freedom from error. Chemical aspects of the subject receive masterly treatment. Perhaps the weakest section is that devoted to the nervous system. G.McC.

MALE AND FEMALE, A STUDY OF THE SEXES IN A CHANGING WORLD. By MARGARET MEAD, Assoc. Curator of Ethnology, Amer. Museum of Nat. Hist. Pp. 477. New York: William Morrow, 1949. Price, $5.00.

A MOST readable and interesting, as well as provocative and informative book, written by an authority in her field. Questions of importance to everyone living in this unsettled age are asked and the answers are sought by a method which is probably new to most of us. The whys and wherefores of human motivation and behavior, especially from the point of view of sex roles, are sought among the cultures of 7 different Pacific peoples and are compared to our own. As a result, the reader obtains a better insight into our own culture, into what we are and how we got that way. W. P.

VISION. By ARNOLD GESELL, M.D., FRANCES L. ILG, M.D., and GLENNA E. BULLIS. Pp. 329; 72 ills. New York: Paul B. Hoeber, 1949. Price, $6.50.

THE Authors have made a systematic investigation of the growth of the child's visual functions as determined by the behavior patterns, graded tests of visual skills and objective determinations of the child's refraction. The questions which they have set out to answer are: How does the child use his eyes at different ages? When does he show visual competence in such things as the handling of toys, tools, and eating utensils? Do the eyes work together coordinately; and, if not, when is his strabismus generally resolved? How does his visual behavior comport with his general behavior? Do we need a broader kind of visual hygiene to protect the growing eyes of today?

The method employed has been to photograph children in a specially designed photographic dome encased in a one-way vision

screen which conceals the observer from the infant but permits full view of the infant's behavior. The book will be valuable for pediatricians as well as for ophthalmologists. Most of the Authors' conclusions agree well with those in standard ophthalmologic texts. F. A.

A HISTORY OF OTO-LARYNGOLOGY. By R. SCOTT STEVENSON, M.D., and DOUGLAS GUTHRIE, M.D., Edinburgh. Pp. 155; 52 ills. Balt.: Williams & Wilkins, 1949. Price, $5.00.

ALTHOUGH numerous articles in medical journals and a few chapters in books have dealt with various facets of the history of otology, rhinology and laryngology, this small book appears to be the first history of the specialty to be written. The authors assess the subject as a whole, tracing its rise and progress from the earliest times through the Middle Ages and the Renaissance, the 17th, 18th and 19th centuries, down to the present. The great interest of the oto-rhino-laryngologic specialty is that it still contains many fascinating and stimulating problems for its practitioners. Curious-minded readers will be amply rewarded in a quest for background by mining the pages of history. N. F.

PRACTICAL OFFICE GYNECOLOGY. By KARL JOHN KARNAKY, M.D., Asst. Prof. of Clinical Gynecology, Baylor Univ. Pp. 261; 113 ills. Springfield, Ill.: Charles C Thomas, 1947. Price, $7.50.

THIS book was designed to give practical help in the office management of patients with gynecological disorders. It is well printed and handsomely illustrated. The text is a personalized expression of a clinician of wide experience. The material dealing with the physiology and infections of the lower genital tract is excellent. Female endocrinology and functional menstrual disorders are well presented, but this Reviewer doubts that most gynecologists share the Author's enthusiasm for estrogen therapy. No mention is made of the means for the detection of early carcinoma of the cervix, nor of the use of pessaries in the management of pelvic relaxation.

Physicians seeing female patients will find the book practically useful. P. K.

NEW BOOKS

Children with Mental and Physical Handicaps. By J. E. WALLACE WALLIN, Ph.D. Pp. 549; 34 ills. New York: Prentice-Hall, 1949. Price, $6.65.

WRITTEN by a man with 40 years experience with handicapped children, this book serves as an excellent reference book in its field. It gives information of value to workers in special education and child guidance, to pediatricians, psychologists, psychiatrists, and social workers. It tells (among many things) about mental defectives, backward children, psychopaths, endocrinopathic disorders, cretinism, mongolism, dwarfs, epilepsy, orthopedic disabilities such as cerebral palsy and poliomyelitis, and cerebral syphilis. W. P.

Die Naturgemässe Heilweise. Von Dr. Med. ADOLF HOFF. Pp. 295; 22 ills. Stuttgart: Marquardt & Cie., 1949. Price, D.M. 18.50.

Medical Clinics of North America. Mass. Genl. Hospital Number. Pp. 1211-1498. Phila.: W. B. Saunders, September, 1949. Price, $15.00 a year.

THIS number is featured by a symposium on recent advances in medicine, consisting of 16 contributions by members of the staff of the Massachusetts General Hospital. Included are discussions of radioactive iodine in thyroid disease, the diagnosis and treatment of myxedema, thyrotoxicosis and nodular goiter; cardiac arrythmias and neurocirculatory asthenia; hypertension in children and in adults; pernicious anemia; rheumatoid arthritis; the use of nitrogen mustards; the "dumping" syndrome, and gastritis. There are also 5 miscellaneous contributions on diabetes, ulcerative colitis, headache, intestinal obstruction and infections of the urinary tract by clinicians from other hospitals in New York, Chicago and Philadelphia. E. R.

Weitere Fortschritte in der Blutgerinnungslehre. Von Prof. Dr. med. KARL LENGGENHAGER, Univ. Bern. Pp. 243; 65 ills. Stuttgart: Georg Thieme Verlag (New York: Grune & Stratton), 1949. Price, $5.50.

THE format of this book on blood coagulation is interesting; headings are in the form of direct questions which are then answered with the aid of experimental evidence. Separate chapters are devoted to the actions of Prothrombokinin, Thrombokinin, Prothrombin, Thrombin, and Fibrinogen. In a discussion of hemophilia, the Author agrees with the work of Tocantins and Quick. A short chapter on Heparin is well written. The book capably covers fundamentals and introduces some original concepts. It is an excellent monograph embodying the Author's 18 years of experience in the field of blood coagulation. G. B.

Psychoanalytic Study of the Child. Vol. III/IV. Edited by ANNA FREUD, *et al.* Pp. 493. New York: International Universities Press, 1949. Price, $10.00.

THIS book contains 25 articles on important subjects written by leaders in the field of psychoanalysis of children. It is intended for psychiatrists but will be of interest to all those who are interested in the development of children or in problems of childhood. W. P.

More About Psychiatry. By CARL BINGER, M.D. Pp. 201. Chicago: Univ. of Chicago Press, 1949. Price, $4.00.

FOURTEEN reprinted essays which entertainingly illustrate emotional and psychiatric problems and an investigator's views on the world situation today.

Homöopathie und Harmonische Ordnung der Heilkunde. Von Prof. Dr. Med. AUGUST BIER. Pp. 268. Stuttgart: Marquardt & Cie., 1949. Price, D.M. 19.50.

Migraine Cervicale. Von Dr. med. et Dr. sc. biol., WERNER BÄRTSCHI-ROCHAIX, Univ. Bern. Pp. 188; 51 ills. Bern: Hans Huber; imported by Grune & Stratton, 1949. Price, S.fr. 21.80.

An Introduction to Cardiology. By GEOFFREY BOURNE, M.D., F.R.C.P., St. Bartholomew's Hospital. Pp. 264; 65 ills. Balt.: Williams & Wilkins, 1949. Price, $4.50.

Symposium on Medicolegal Problems. Series 2. Edited by SAMUEL LEVINSON, M.D., Ph.D. Pp. 276; 44 ills. Phila.: J. B. Lippincott, 1949. Price, $5.00.

Rational Medicine. By JOHN W. TODD, M.D. (Lond.), M.R.C.P. (Lond.). Pp. 378. Balt.: Williams & Wilkins, 1949. Price, $6.50.

Industrial Toxicology. By LAWRENCE T. FAIRHALL, Chief, Industrial Hygiene Laboratory, Industrial Health Division, Public Health Service. Pp. 483. Balt.: Williams & Wilkins, 1949. Price, $6.00.

NEW EDITIONS

Oral Histology and Embryology. Edited by BALINT ORBAN, Univ. of Illinois. 2d ed. Pp. 364; 265 ills., 4 in color. St. Louis: C. V. Mosby, 1949. Price, $8.00.

Diseases of the Nervous System. By F. M. R. WALSHE, M.D., Univ. College, London. 6th ed. Pp. 359; 60 ills. Balt.: Williams & Wilkins, 1949. Price, $5.00.

THIS masterpiece of medical writing, which describes in the simplest terms for "practitioners and students" the commonest diseases of the nervous system, now appears in its 6th edition since 1940. The chapter on neurosyphilis has incorporated the work on penicillin, "a new therapeutic agent of the first importance". The discussion of poliomyelitis and abscess are enlarged, but it seems unfortunate that no mention is made of the arthropod-borne encephalitides. G. G.

Outlines of Internal Medicine. Edited by C. J. WATSON, M.D., Univ. of Minnesota. 6th ed. Pp. 443 + 94. Dubuque, Iowa: Wm. C. Brown, 1949. Price, $12.00.

THE well known outlines of the third year medical lectures at the University of Minnesota have now been published in a single volume. These are a concise presentation of the most significant information about medical diseases by a group of well qualified teachers and clinicians. The substitution of this outline for didactic lectures and the use of the hour for case demonstration instead is a further example of the present trend in medical teaching in the United States. The bibliography at the end of each lecture should prove useful to students. An excellent section on clinical chemistry and microscopy is presented at the end of the book. It is not designed for the use of anyone except medical students and without an index can not be easily used as a reference book. A. R.

Human Helminthology. By ERNEST CARROLL FAUST, Ph.D., Prof. of Tropical Diseases and Hygiene, Tulane Univ. of Louisiana. 3d ed. Pp. 744; 313 ills. Phila.: Lea & Febiger, 1949. Price, $10.00.

MILITARY operations in tropical countries during the interval between the publication of the 2d and 3d editions of this book have necessitated intensive study of the animal parasites which contribute so much to morbidity of populations native to these regions. Professor Faust has incorporated the results of these and other studies of this period in this edition, adding much new material on Anthelmintics, a new chapter on Hirudinea (leeches), and 34 illustrations. The bibliography has been increased accordingly. As a result of utilizing a larger page, the thickness of the book is not changed materially; it continues to be an outstanding reference work. H. R.

Atlas of Obstetric Technic. By PAUL TITUS, M.D. 2d ed. Pp. 197; 203 ills. St. Louis: C. V. Mosby, 1949. Price, $7.50.

THIS atlas has had a well deserved popularity since its first printing in 1943. The pictorial form and concise running text have been retained in the present edition. Improvements include rearrangement of subjects, addition of several new sections, and revision of the descriptive reading matter. P. K.

The Eye and its Diseases. Edited by CONRAD BERENS, M.D., F.A.C.S. 2d. ed. Pp. 1092; 436 figs., 8 in color. Phila.: W. B. Saunders, 1949. Price, $16.00.

A Manual of Physical Therapy. By RICHARD KOVACS, M.D., Prof. of Physical Medicine. 4th ed. Pp. 328; 124 ills. Phila.: Lea & Febiger, 1949. Price, $3.75.

The Science and Art of Joint Manipulation. (Vol. I. *The Extremities*). By JAMES MENNELL, M.D., Consulting Physician in Physical Medicine, St. Thomas's Hospital. 2d ed. Pp. 215; 299 ills. Phila.: Blakiston, 1949. Price, $7.50.

Injuries of the Brain and Spinal Cord and their Coverings. Edited by SAMUEL BROCK, M.D., New York Univ. 3d ed. Pp. 783; 124 figs. Balt.: Williams & Wilkins, 1949. Price, $10.00.

THIS edition has greatly benefited from the experiences of the war. Of the original 23 chapters, 14 have been thoroughly revised and enlarged. Four new chapters have been added (injuries of the vertebral column; herniation and protrusion of the intravertebral disc; war neuroses following head and brain injuries; the effects of electric shock on the nervous system). F. L.